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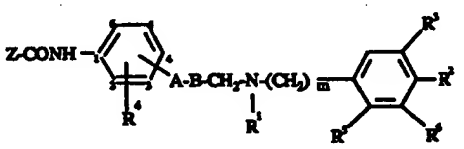
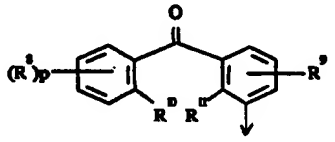
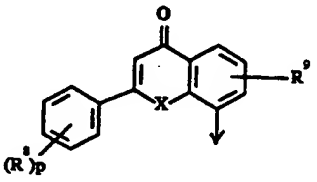
(54) Title: ANILIDE DERIVATIVES



(I)



(a)



(b)

(57) Abstract

Compounds are described of general formula (I) and salts and solvates thereof, including physiologically acceptable salts and solvates thereof, in which: Z represents either Het, (a), or (b); Het represents an optionally substituted bicyclic or tricyclic ring selected from quinolin-4-yl, isoquinolin-1-yl, isoquinolin-3-yl, quinolin-3-yl, quinolin-2-yl, quinoxalin-2-yl, naphthalen-1-yl, naphthalen-2-yl, indol-2-yl, 4-oxo-4H-1-benzopyran-2-yl, phenazin-1-yl and phenothiazin-1-yl or an aryl substituted monocyclic ring selected from 2-aryl-4-thiazolyl, 2-aryl-5-thiazolyl, 5-aryl-2-thienyl, 2-aryl-4-triazolyl and 1-aryl-4-pyrazolyl where aryl represents a phenyl or pyridyl ring optionally substituted by a halogen atom or a trifluoromethyl, C_{1-4} alkyl or C_{1-4} alkoxy group. The above-mentioned bicyclic or tricyclic rings may be unsubstituted or substituted by one, two or three groups selected from C_{1-4} alkyl and C_{1-4} alkoxy. Quinolin-4-yl rings may also be substituted in the ring 2 position by phenyl or phenyl substituted by C_{1-4} alkoxy. Indol-2-yl rings may also be substituted in the ring 3 position by benzoyl; R^8 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylthio, amino or nitro group; p represent 1; or when R^8 represents C_{1-4} alkoxy p may also represent 2 or 3; R^9 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group; R^{10} and R^{11} may each represent a hydrogen atom or together form a bond or a linking atom selected from -O- or -S-; and X represents an oxygen atom or NR^{12} (where R^{12} represents a hydrogen atom or a C_{1-4} alkyl group). The novel compounds of formula (I) can sensitize multi-drug resistant cancer cells to chemotherapeutic agents and may be formulated for use in therapy, particularly to improve or increase the efficacy of an anti-tumour drug.

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ANILIDE DERIVATIVES

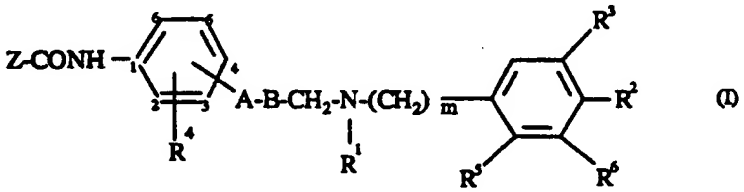
This invention relates to anilide derivatives, to processes for their preparation, to pharmaceutical compositions containing them, and to their medical use. In particular it relates to compounds and compositions which are capable of sensitizing multidrug-resistant cancer cells to chemotherapeutic agents.

In many patients, the efficacy of cancer chemotherapy is initially poor or decreases after initial treatment due to the development of resistance to anticancer drugs, known as multidrug-resistance. Multidrug-resistance is a process whereby malignant cells become resistant to structurally diverse chemotherapeutic agents following treatment with a single anti-tumour drug. This acquired drug resistance can be a major clinical obstacle in the treatment of cancer. Some tumours are intrinsically multidrug-resistant, and hence do not respond to chemotherapy.

It has been shown that this type of resistance can be reversed by some calcium channel blockers such as nicardipine and verapamil, by antiarrhythmic agents such as amiodarone and quinidine, as well as by natural products such as cepharanthine. However, these compounds exert their multidrug resistant cell sensitizing activity only at very high doses, well above their intrinsic toxic level, and this severely limits their clinical use in the field of cancer chemotherapy.

A novel group of compounds has now been found which can sensitize multidrug-resistant cancer cells to chemotherapeutic agents at dose levels at which these novel compounds show no toxicity.

Thus, the present invention provides a compound of formula (I):



(I)

and salts and solvates thereof, including physiologically acceptable salts and solvates thereof, in which:

A represents an oxygen or a sulphur atom, a bond or a group $(CH_2)_lNR^7$ (where l represents zero or 1 and R^7 represents a hydrogen atom or a methyl group);

B represents a C_{1-4} alkylene chain optionally substituted by a hydroxyl group, except that the hydroxyl group and moiety A cannot be attached to the same carbon atom when A represents an oxygen or sulphur atom or a group $(CH_2)_lNR^7$, or when A represents a bond B may also represent a C_{2-4} alkenylene chain;

R^1 represents a hydrogen atom or a C_{1-4} alkyl group;

m represents 1 or 2;

R^2 represents a hydrogen or a halogen atom, or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group;

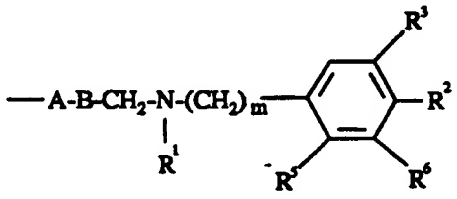
R^3 represents a hydrogen atom or a C_{1-4} alkoxy group;

R^4 represents a hydrogen atom or a C_{1-4} alkyl or C_{1-4} alkoxy group;

R^5 represents a hydrogen atom or R^1 and R^5 together form a group $-(CH_2)_n-$ where n represents 1 or 2;

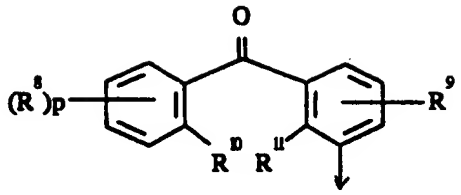
R^6 represents a hydrogen atom or a C_{1-4} alkoxy group;

the group

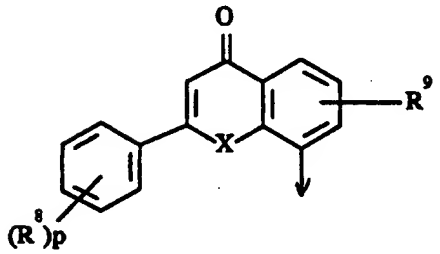


is attached at the benzene ring 3 or 4 position relative to the carboxamide substituent, provided that when the group is attached at the benzene ring 3 position then R^4 must b attached at the benzene ring 6 position; and

Z represents either Het,



or



;

Het represents an optionally substituted bicyclic or tricyclic ring selected from quinolin-4-yl, isoquinolin-1-yl, isoquinolin-3-yl, quinolin-3-yl, quinolin-2-yl, quinoxalin-2-yl, naphthalen-1-yl, naphthalen-2-yl, indol-2-yl, 4-oxo-4H-1-benzopyran-2-yl, phenazin-1-yl and phenothiazin-1-yl or an aryl substituted monocyclic ring selected from 2-aryl-4-thiazolyl, 2-aryl-5-thiazolyl, 5-aryl-2-thienyl, 2-aryl-4-triazolyl and 1-aryl-4-pyrazolyl where aryl represents a phenyl or pyridyl ring optionally substituted by a halogen atom or a trifluoromethyl, C_{1-4} alkyl or C_{1-4} alkoxy group. The above mentioned bicyclic or tricyclic rings may be unsubstituted or substituted by one, two or three groups selected from C_{1-4} alkyl and C_{1-4} alkoxy. Quinolin-4-yl rings may also be substituted in the ring 2 position by phenyl or phenyl substituted by C_{1-4} alkoxy. Indol-2-yl rings may also be substituted in the ring 3 position by benzoyl;

R^8 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylthio, amino or nitro group;

p represents 1; or when R^8 represents C_{1-4} alkoxy p may also represent 2 or 3;

R^9 repr sents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group;

R^{10} and R^{11} may each represent a hydrogen atom or together form a bond or a linking atom selected from -O- or -S-; and

X represents an oxygen atom or NR^{12} (where R^{12} represents a hydrogen atom or a C_{1-4} alkyl group).

As used herein, an alkyl group, either as such or as part of an alkoxy or alkylthio group may be a straight chain or branched chain alkyl group, for example a methyl, ethyl or prop-2-yl group.

A halogen substituent may be a fluorine, chlorine, bromine or iodine atom.

The groups represented by R^8 and R^9 may be situated at any available positions in the relevant benzene rings.

Examples of the chain -A-B-CH_2- include -$(CH_2)_2$-, -$(CH_2)_3$-, -$(CH_2)_4$-, -$(CH_2)_5$-, -$CH_2NMe(CH_2)_2$-, -CH=$CHCH_2$-, -CH_2CH=$CHCH_2$-, -CH(OH)CH_2-, -$O(CH_2)_2$-, -$O(CH_2)_3$-, -$OCH_2CH(OH)CH_2$-, -$NH(CH_2)_2$-, -$S(CH_2)_2$- and -$S(CH_2)_3$-.

When R^1 represents a hydrogen atom or a C_{1-4}alkyl group, preferably R^1 represents a C_{1-4}alkyl (e.g. methyl) group.

R^8 preferably represents a hydrogen or fluorine atom or a C_{1-4} alkoxy (e.g. methoxy), C_{1-4} alkyl (e.g. methyl) or C_{1-4} alkylthio (e.g. methylthio) group.

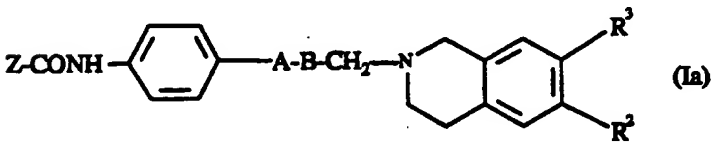
R^9 preferably represents a hydrogen atom or a C_{1-4} alkoxy (e.g. methoxy) group.

A preferred class of compounds of formula (I) is that in which R^2 represents a hydrogen atom or a C_{1-4}alkoxy (e.g. methoxy) group, R^3 represents a hydrogen atom or a C_{1-4}alkoxy (e.g. methoxy) group and R^6 represents a hydrogen atom or a C_{1-4}alkoxy (e.g methoxy) group, provided that at least one of R^2, R^3 and R^6 represents a C_{1-4}alkoxy (e.g. methoxy) group. A particularly preferred class of compounds of formula (I) is that in which R^2 and R^3 each represent a C_{1-4}alkoxy (e.g. methoxy) group and R^6 represents a hydrogen atom.

R^4 preferably represents a hydrogen atom or a methyl, ethyl, methoxy or ethoxy group. Compounds of formula (I) in which R^4 represents a hydrogen atom are particularly preferred.

A preferred group of compounds of formula (I) is that in which m represents 1 and R^1 and R^5 together form a group $-(CH_2)_2-$, and physiologically acceptable salts and solvates thereof.

A particular group of compounds of formula (I) is that of formula (Ia)



(Ia)

wherein Z is as defined in formula (I) above;

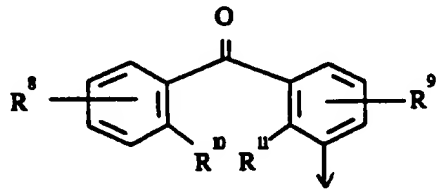
A represents an oxygen or a sulphur atom or a bond;

B represents an unsubstituted C_{1-4} alkylene chain;

R^2 and R^3 each independently represents a C_{1-4} alkoxy group; (eg methoxy); and physiologically acceptable salts and solvates thereof.

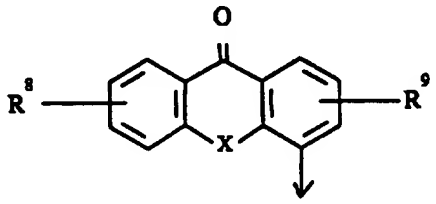
A particular group of compounds of Formula (Ia) are compounds in which Z represents Het as previously defined.

Another particular group of compounds of Formula (Ia) are compounds in which Z represents



wherein R^8 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylthio or nitro group, R^9 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group and R^{10} and R^{11} are as previously defined.

A further particular group of compounds of formula (Ia) are compounds in which Z represents



wherein R^8 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylthio or nitro group, R^9 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group and X represents an oxygen atom or NH.

Particularly preferred compounds of formula (Ia) are those in which R^8 represents a hydrogen or fluorine atom or a C_{1-4} alkoxy (e.g. methoxy) or C_{1-4} alkyl (e.g. methyl) group and R^9 represents a hydrogen atom.

It is to be understood that the present invention includes all combinations of the aforementioned particular and preferred groups.

Suitable physiologically acceptable salts of the compounds of formula (I) include acid addition salts formed with organic or inorganic acids, for example, hydrochlorides, hydrobromides, sulphates, alkyl- or arylsulphonates (e.g. methanesulphonates or p-toluenesulphonates), phosphates, acetates, citrates, succinates, lactates, tartrates, fumarates and maleates. The solvates may, for example, be hydrates.

Other salts which are not physiologically acceptable may be useful in the preparation of compounds of formula (I) and these form a further part of the invention.

The ability of the compounds of formula (I) to sensitize multidrug-resistant cells has been demonstrated in vitro in the multidrug-resistant Chinese hamster ovary cell line (described by Bech-Hansen et al., J. Cell. Physiol., 1976, 88,23-32) and the multidrug-resistant human mammary carcinoma line (described by Batist et al., (J. Biol. Chem., 1986, 261, 1544-1549) using an assay similar to that described by Carmichael et al., Cancer Research, 1987, 47, 936.

The ability of the compounds of formula (I) to sensitize multidrug-resistant cells has also been demonstrated in vivo in the tumour line P388R (described by Johnson et al., Cancer Treat. Rep., 1978, 62, 1535-1547). The methodology used is similar to that described by Boesch et al., Cancer Research, 1991, 51, 4226-4233. However, in our study the compounds were administered orally, intravenously or intraperitoneally in a single dose.

The present invention accordingly provides a compound of formula (I) or a physiologically acceptable salt or solvate thereof for use in therapy, more particularly for use in the treatment of a mammal, including a human, which is suffering from cancer to :

(a) improve or increase the efficacy of an antitumour drug; or

(b) increase or restore sensitivity of a tumour to an antitumour drug; or

(c) reverse or reduce resistance, whether acquired, induced or inate, of a tumour to an antitumour drug.

The present invention also provides a method of treatment of a mammal, including a human, which is suffering from cancer, which method comprises administering to said mammal an effective amount of a compound of formula (I) or a physiologically acceptable salt or solvate thereof to :

(a) improve or increase the efficacy of an antitumour drug; or

(b) increase or restore sensitivity of a tumour to an antitumour drug; or

(c) revers or reduc resistance, whether acquir d, induced or inate, of a tumour to an antitumour drug.

In another aspect, the present invention provides the use of a compound of formula (I) or a physiologically acceptable salt or solvate thereof for the manufacture of a medicament for the treatment of a mammal, including a human, which is suffering from cancer to :

(a) improve or increase the efficacy of an antitumour drug; or

(b) increase or restore sensitivity of a tumour to an antitumour drug; or

(c) reverse or reduce resistance, whether acquired, induced or inate, of a tumour to an antitumour drug.

It will be appreciated that the compounds according to the present invention are administered in conjunction with an antitumour drug. Thus, in a further aspect, the present invention provides a product containing a compound of formula (I) or a physiologically acceptable salt or solvate thereof and an antitumour drug as a combined preparation for simultaneous, separate or sequential use in treating cancer, more particularly to :

(a) improve or increase the efficacy of said antitumour drug; or

(b) increase or restore sensitivity of a tumour to an antitumour drug; or

(c) reverse or reduce resistance, whether acquired, induced or inate, of a tumour to an antitumour drug.

Examples of suitable antitumour drugs for use in conjunction with compounds of the present invention include Vinca alkaloids (e.g. vincristine, vinblastine and vinorelbine), anthracyclines (e.g. daunorubicin, doxorubicin and aclarubicin), taxol and derivatives thereof (e.g. taxotere), podophyllotoxins (e.g. etoposide and VP16), mitoxantrone, actinomycin, colchicine, gramicidine D, amsacrine or any drug having cross-resistance with the above drugs characterised by the so-called MDR phenotype.

It will be appreciated that if administration of the two drugs is not simultaneous, the delay in administering the second of the active ingredients should not be such as to lose th beneficial effect of the combination.

Thus, in a further aspect, the present invention provides a compound of formula (I) or a physiologically acceptable salt or solvate thereof and an anticancer drug in the presence of each other in the human or non-human animal body for use in treating cancer, more particularly to :

(a) improve or increase the efficacy of said antitumour drug; or

(b) increase or restore sensitivity of a tumour to an antitumour drug; or

(c) reverse or reduce resistance, whether acquired, induced or inate, of a tumour to an antitumour drug.

Some tumours are often intrinsically multidrug-resistant, notably colon carcinomas, renal cell carcinomas, hepatomas and adrenocortical carcinomas.

Other types of tumour are often initially sensitive but can become multidrug-resistant, notably leukaemias, lymphomas, myelomas, paediatric tumours (e.g. neuroblastomas), sarcomas, and breast, ovarian and lung cancers.

Hence the compounds of the invention are particularly useful in the treatment of mammals, including humans, receiving chemotherapy for one of the above types of cancer.

In using a compound of formula (I) or a physiologically acceptable salt or solvate thereof and an antitumour drug it may be preferable to employ the active ingredients in the form of separate pharmaceutical formulations, although a single combined formulation can be used as demonstrated hereinafter. However, in the latter formulation both active ingredients must of course be stable and mutually compatible in the particular formulation employed.

Pharmaceutical formulations of suitable antitumour drugs and appropriate dosages and dosage rates will generally correspond with those one would use if administering the antitumour drug alone to treat a tumour.

Suitable pharmaceutical formulations and appropriate dosages and dosage rat s of compounds of formula (I) and physiologically acceptable salts and solvates thereof are described hereinafter.

Thus, in a further aspect, the invention provides a pharmaceutical composition which comprises a compound of formula (I) or a physiologically acceptable salt or solvate thereof together with one or more physiologically acceptable carriers or excipients.

In another aspect, the present invention provides a pharmaceutical composition which comprises an active amount of a compound of formula (I) or a physiologically acceptable salt or solvate thereof for use in the treatment of a mammal which is suffering from cancer, to :

(a) improve or increase the efficacy of an antitumour drug; or

(b) increase or restore sensitivity of a tumour to an antitumour drug; or

(c) reverse or reduce resistance, whether acquired, induced or inate, of a tumour to an antitumour drug.

The compounds according to the invention may be formulated for oral, buccal, parenteral or rectal administration, of which oral and parenteral are preferred.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. sodium lauryl sulphate or sodium starch glycolate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g. sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g. lecithin or acacia); non-aqueous vehicles (e.g. almond oil, oily esters, ethyl alcohol or fractionated v getable oils); and preservatives (e.g. methyl or propyl-p-hydroxybenzoates or

sorbic acid). The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds of the invention may be formulated for parenteral administration by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form e.g. in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily, aqueous or alcoholic vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

The compounds of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

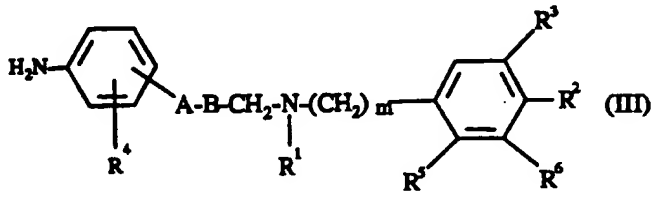
A proposed daily dose of the compounds of the invention for administration to a human (of approximately 70kg body weight) is about 10mg to 1000mg, more preferably about 25mg to 500mg. It will be appreciated that it may be necessary to make routine variations to the dosage, depending on the age and condition of the patient, and the route of administration. For example, a daily dose of about 1mg/kg may be appropriate for administration to a human by infusion. The daily dose may be given as a single unit or as two or more subunits administered after appropriate time intervals.

Compounds of general formula (I) and physiologically acceptable salts and solvates thereof may be prepared by the general methods outlined hereinafter. In the following description, the groups Z, R^1 to R^6, m, A and B are as defined for compounds of formula (I) unless otherwise specified.

Thus according to a first general process (A), a compound of formula (I) may be prepared by reacting a compound of formula (II):

$$Z\text{-}CO_2H \quad (II)$$

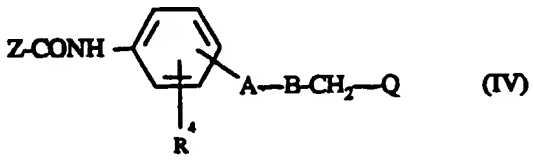
with a compound of formula (III)



(III)

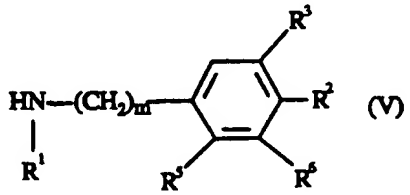
The reaction may be effected using a coupling reagent standardly used in peptide synthesis, such as dicyclohexylcarbodiimide (optionally in the presence of 1-hydroxybenzotriazole), diphenylphosphoryl azide or N,N'-carbonyldiimidazole. The reaction may be conveniently effected in an inert solvent such as an ether (e.g. tetrahydrofuran), a halogenated hydrocarbon (e.g. dichloromethane), an amide (e.g. dimethylformamide) or a ketone (e.g. acetone), and at a temperature of, for example, -10 to +100°C, more preferably at about room temperature.

According to another general process (B), a compound of formula (I) may be prepared by reacting a compound of formula (IV):



(IV)

wherein Q represents a halogen (e.g. bromine) atom, with a compound of formula (V):

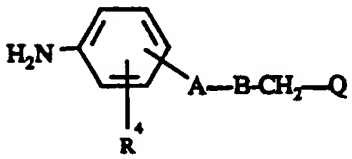


(V)

or a salt thereof. The reaction may be effected in the presence of an acid acceptor such as an alkali metal carbonate (e.g. potassium carbonate), in the presence or absence of a solvent, at an elevated temperature (e.g. 50 to 120°C). Suitable solvents include ketones (e.g. acetone, methylethylketone or methylisopropylketone) and alcohols (e.g. ethanol or isopropanol).

Compounds of formula (III) may be prepared according to the methodology described in published European Application 0494623.

Compounds of formula (IV) may be prepared by the reaction of a compound of formula (II) as defined previously, with a compound of formula (VI):



(VI)

wherein Q represents a halogen (e.g. bromine) atom, under the conditions described in process (A) above for the reaction of a compound of formula (II) with a compound of formula (III).

Intermediates of formula (IV) are novel compounds and represent a further aspect of the present invention.

Compounds of formula (II) are either known in the art or may be prepared by conventional methods, for example as described in the Examples section hereinafter.

Compounds of formulae (V) and (VI) are either known in the art or may be prepared according to the methodology described in published European Application 0494623.

Where it is desired to isolate a compound of the invention as a salt, for example a physiologically acceptable salt, this may b achieved by reacting the compound of formula (I) in the form of the free base with an appropriate acid, preferably with an quivalent amount, in a suitable solvent such as an alcohol (.g. ethanol or methanol), an aqueous alcohol (e.g. aqueous thanol), a

halogenated hydrocarbon (e.g. dichloromethane), an ester (e.g. ethyl acetate) or an ether (e.g. tetrahydrofuran), or a mixture of two or more of such solvents.

Physiologically acceptable salts may also be prepared from other salts, including other physiologically acceptable salts, of the compound of formula (I) using conventional methods.

The invention is further illustrated by the following Intermediates and Examples which are not intended to limit the invention in any way. All temperatures are in °C. ^1H NMR spectra were obtained for dilute solutions in $CDCl_3$ unless otherwise stated. Solvents were dried , where indicated, over sodium sulphate. Silica gel used for column chromatography was Merck 60, 230-400 mesh. The following abbreviatons are used: THF - tetrahydrofuran; DMF - dimethylformamide.

Intermediate 1

Ethyl 3,4-dihydro-6-methoxy-3-oxo-2-quinoxalinecarboxylate

2-amino-4-methoxyaniline (25g) triethylamine (25.4 ml) and ethanol (250 ml) were stirred under nitrogen at 5°. Diethyl bromomalonate (40.1 ml) in ethanol (50 ml) was added dropwise over 30 min. The mixture was stirred at 5° for 30 minutes. After 16 hours at room temperature, the precipitate was filtered off and stirred in water (800 ml) containing 1N hydrochloric acid (100 ml) for 1 hour. The mixture was filtered. The residue was washed with water and dried in vacuo to give the title compound (15.3 g) as a solid, mp : 227°.

Intermediate 2

(a) Ethyl 3-chloro-6-methoxy-2-quinoxalinecarboxylate

Phosphorous oxychloride (46 ml) was added to Intermediate 1 (10g). The mixture was heated at 100° for one hour, allowed to cool, and then carefully poured into ice (800 g). The pH of this mixture was adjusted to 3 by addition of aqueous ammonia. The resulting yellow solid was filtered off, washed with water, and recrystallised from aqueous acetone to give the title compound (10.08 g) as a solid, mp = 75°.

The following compound was prepared in a similar manner :

(b) Ethyl 3-chloro-6,7-dimethyl-2-quinolaxinecarboxylate

The title compound (10.7 g) was obtained as a solid, mp = 115° from ethyl 3,4-dihydro-3-oxo-6,7-dimethyl-2-quinoxalinecarboxylate * (10 g).

* Chem. Abstracts 41, 3469c.

Intermediate 3

(a) 3-Methoxy-6,7-dimethyl-2-quinoxalinecarboxylic acid

Intermediate 2(b) (2g) was added to a solution of sodium (0.43g) in dry methanol (100 ml). The solution was refluxed for 1 hour, cooled to room temperature and water (20 ml) was added. The solution was refluxed for 1 hour. The cool solution was filtered off. The filtrate was acidified to pH 3 with 2N hydrochloric acid. The product crystallised and was then filtered, washed with water and dried in vacuo to give the title compound (1.59g) as a solid, mp = 180 - 182°.

The following compound was prepared in a similar manner :

(b) 3-Ethoxy-6,7-dimethyl-2-quinoxalinecarboxylic acid

The title compound (0.88g) was obtained as a solid, mp = 116°, from Intermediate 2(b) (1.3g) in ethanol.

Intermediate 4

Ethyl 6-methoxy-3-ethylthio-2-quinoxalinecarboxylate

To a suspension of sodium hydride (1.8g) in THF was added a solution of ethanethiol in dry THF (30 ml). After 15 min, a solution of Intermediate 2(a) (10g) in dry THF (50 ml) was added. The mixture was stirred at room temperature for 16 hours. The precipitate was filtered off and the filtrate was evaporated. The residu was extracted with dichloromethane, washed with water, dried, concentrated in vacuo and recrystallised from isopropanol (50 ml), to give the titl compound (5g) as a solid, mp = 70°.

Intermediate 5

Ethyl 6-methoxy-2-quinoxalinecarboxylate

To a solution of Intermediate 4 (5g) was carefully added Raney nickel (80g). The mixture was stirred at room temperature for 1 hour. The Raney nickel was filtered off and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with cyclohexane:ethylacetate (70 : 30) to give the title compound (2.5 g) as a solid.

NMR includes δ 1.48 (3H,t,CH_3); 3.84(3H,s,OCH_3); 4.57(2H,q,CH_2).

Intermediate 6

6-Methoxy-2-quinoxalinecarboxylic acid

To a solution of Intermediate 5 (2.5g) in ethanol (60ml) was added an aqueous solution of 30% sodium hydroxide. The mixture was refluxed for 30 minutes. After evaporation, the mixture was acidified by addition of 1N hydrochloric acid. The white crystals were filtered off and dried to give the title compound (2 g) as a solid, mp = 248°.

Intermediate 7

2-Methoxy-3'-methylbenzophenone

A mixture of 2-methoxybenzonitrile (4.3 ml) and the Grignard reagent of m-bromotoluene (6.6 g) in ether was refluxed for 1h and hydrolysed with dilute hydrcchloric acid with heating. The aqueous layer was then extracted with ether, and the resultant organic layer was dried and evaporated to give the title compound (5.5 g) as an oil.

Intermediate 8

3-(2-Methoxybenzoyl)benzoic acid

A solution of Intermediat 7 (5.4 g) in a mixture of pyridine (50 ml) and water (70 ml) was heated to 50° and treated dropwise with potassium permanganate (19g). The mixture was then refluxed for 2 h, cool d to room t mperature,

filtered and the salts were washed with hot water. The aqueous solution was then acidified with sulphuric acid and extracted with dichloromethane. The organic layer was then dried and evaporated to give the title compound (4.4g) as a solid, mp = 170-172°.

Intermediate 9

(a) 1-(3-Bromopropoxy)-3-methoxy-4-nitrobenzene

A mixture of 3-methoxy-4-nitrophenol (Intermediate 18 in EP-A-494623) (2.4g), 1,3-dibromopropane (7.5ml) and potassium carbonate (2.2g) in DMF (30ml) was stirred at room temperature for 24h. The mixture was filtered and the filtrate was evaporated to dryness. The residue was treated with water and extracted with dichloromethane. The organic extract was then washed with 5% sodium hydroxide solution and brine, dried and concentrated in vacuo to give the title compound (3.5g) as an oil.

NMR includes δ 2.3 (2H,m,CH_2), 3.6 (2H,t,CH_2Br), 3.8 (3H,s,OCH_3), 4.1 (2H,t, CH_2O).

The following compounds were prepared in a similar manner to Intermediate 9 (a):

(b) 1-(4-Bromobutoxy)-4-nitrobenzene

The title compound was obtained from 4-nitrophenol and 1,4-dibromobutane.

NMR includes δ 4.01 (2H,m,CH_2Br), 3.4 (2H,m,CH_2Ar).

(c) 1-(3-Bromopropoxy)-3-methyl-4-nitrobenzene

The title compound (33g) was obtained as an oil from 3-methyl-4-nitrophenol (25g) and 1,3-dibromopropane (83ml).

NMR includes δ 2.3 (2H,m,CH_2), 2.5 (3H,s,CH_3), 3.6 (2H,t,CH_2Br), 4.1 (2H,t,OCH_2).

Intermediate 10

(a) 1,2,3,4-Tetrahydro-6,7-dimethoxy-2-[3-(3-methoxy-4-nitrophenoxy)propyl]isoquinoline

A mixture of Intermediate 9(a) (0.7g), 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (0.4g) and potassium carbonate (0.36g) in DMF (25ml) was heated at 60° for 16h. The mixture was filtered and the filtrate was evaporated. The residue was treated with water and extracted with dichloromethane. The organic layer was dried, concentrated, and the resultant residue was purified by column chromatography eluting with dichloromethane:methanol (99:1) to give the title compound (0.64g) as an oil.

NMR includes δ 3.8 (9H,2s, 3 X OCH_3).

The following compounds were prepared in a similar manner to Intermediate 10(a):

(b) 1,2,3,4-Tetrahydro-6,7-dimethoxy-2-[4-(4-nitrophenoxy)butyl]isoquinoline

The title compound was obtained from Intermediate 9(b) and 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline.

NMR includes δ 3.7(2H,s,NCH_2Ar), 3.9(2H,t,OCH_2).

(c)1,2,3,4-Tetrahydro-6,7-dimethoxy-2-[3-(3-methyl-4-nitrophenoxy)propyl]isoquinoline

The title compound (5.3g) was obtained as an oil from Intermediate 9(c) (5.7g) and 1,2,3,4- tetrahydro-6,7-dimethoxyisoquinoline (4.0g).

NMR includes δ 2.5 (3H,s,CH_3), 3.8 (6H,s, 2 X OCH_3)

(d) N-Methyl-N-(4-nitrobenzyl)veratrylamine

The title compound was obtained as an orange oil from 4-nitrobenzylbromide and N-methylveratrylamine.

NMR includes δ 3.8 (6H, s, 2 x OCH_3), 2.2 (3H, s, NCH_3), 3.65 (2H, s, $NCH_2C_6H_1NO_2$-p), 3.5(2H, s, $NCH_2C_6H_3(OCH_3)_2$).

(e) N-Methyl-N-[3-(4-nitrophenoxy)propyl]benzylamine

The title compound was obtained as the hydrochloride salt (from diethyl ether) from 1-(3-bromopropoxy)-4-nitrobenzene and N-methylbenzylamine. mp = 170-172°.

Intermediate 11

(a) 2-Methoxy-4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine

A solution of Intermediate 10(a) (0.64g) in ethanol (25ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% palladium on carbon (60mg). After hydrogen absorption was completed, the catalyst was filtered off and the solution was concentrated in vacuo to give the title compound (0.4g) as a solid.

NMR includes δ 3.8 (9H,s, 3 X OCH_3), 3.0 (2H,bs,NH_2).

The following compounds were prepared in a similar manner to Intermediate 11(a):

(b) 4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-isoquinolinyl)butoxy]benzenamine

The title compound was obtained from Intermediate 10(b), mp = 114°.

(c) 2-Methyl-4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine

The title compound (4.8g) was obtained as an oil (which subsequently crystallised) from Intermediate 10 (c) (5.3g).

NMR includes δ 2.1 (3H,s,CH_3), 3.8 (6H,s, 2 X OCH_3).

(d) N-(4-Aminobenzyl)-N-methylveratrylamine

The title compound was obtained as a yellow oil from Intermediate 10(d).

NMR includes δ 3.75 (s, 6H 2 X OCH_3), 3.5(4H, 2 X NCH_2Ph), 2.1(3H, s, NCH_3).

(e) 4-[3-(N-methylbenzylamino)propoxyaniline

The title compound was obtained as an oil from Intermediate 10(e). NMR includes δ 3.9 (t, 2H, O-CH_2), 3.4(s, 2H, CH_2Ph), 2.1(t, 2H, N-CH_2), 2.0(s, 3H, N-CH_3), 1.85(m, 2H, CH_2).

Intermediate 12

1-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-3-(4-nitrophenoxy)-2-propanol

A mixture of 1,2-epoxy-3-(4-nitrophenoxy)propane (4g) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (5.4g) in isopropanol (100ml) was heated under reflux for 3h and evaporated. The residue was purified by column chromatography to give the title compound (7.6g) as a yellow oil which solidified on standing.

Intermediate 13

1-(4-Aminophenoxy)-3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-2-propanol

A solution of Intermediate 12 (4g) in ethanol (100ml) was hydrogenated at room temperature in the presence of 10% palladium on carbon (0.4g). After the hydrogen absorption was completed, the catalyst was filtered off and the filtrate concentrated in vacuo to give the title compound (3.5g) as an off white solid, mp = 106°.

Intermediate 14

3-(3-Methoxybenzoyl)benzoic acid

A solution of 3-methoxy-3'-methylbenzophenone* (8 g) in a mixture of pyridine (50 ml) and water (100 ml) was heated to 50° and treated dropwise with potassium permanganate (22 g). The mixture was then refluxed for 12 h, cooled to room temperature, filtered and the salts washed with hot water. The aqueous solution was then acidified with sulphuric acid and the resultant solid was filtered off and recrystallised from a mixture of ethanol/water to give the title compound (5.8 g) as a solid, mp : 160°.

* W.E. Bachmann and J.W. Ferguson, J.A.C.S., 56, 2081-4 (1934).

Intermediate 15

3-(4-Fluorobenzoyl)benzoic acid

A suspension of 4'-fluoro-3-methylbenzophenone* (1.8 g) in water (70 ml) was treated dropwise with potassium permanganate (5.3 g) and the mixture was refluxed for 12 h. After cooling to room temperature, the salts were filtered and washed with hot water. The aqueous solution was then acidified with concentrated hydrochloric acid and the resultant solid was filtered off and dried to give the title compound (1.2 g) as a solid, mp : 180°.

* A. Allais et al., Eur. J. Med. Chem.- Chemica therapeutica, 9, n4, p 381-389 (1974).

Intermediate 16

Methyl 5-(3-fluorobenzoyl)-2-methoxybenzoate

Aluminium trichloride (16.2 g) and 3-fluorobenzoyl chloride (7.5 ml) were added to 1,2-dichloroethane (120 ml) at room temperature. The mixture was cooled to - 5° and salicylic acid (8.3 g) was added portionwise and the mixture was heated to 40°. After 12 h at 40°, the mixture was cooled, poured into ice and acidified with 2N hydrochloric acid. Extraction with ethyl acetate and evaporation gave a white solid. A portion (10 g) of the solid was dissolved in dimethylsulphoxide (60 ml) and potassium carbonate (16 g) was added. After 1 h at room temperature, iodomethane (9.6 ml) was added and the mixture was heated at 40° for 3 h. After cooling, the mixture was poured in to ice and the precipitate was purified by chromatogaphy eluting with toluene/ethyl acetate (90/10) to give the title compound (7 g) as a solid, mp : 140°.

Intermediate 17

N-Benzyl-N-methyl-2-(4-nitrophenoxy)acetamide

MP 95-96°. Prepared from (4-nitrophenoxy)acetic acid and N-methylbenzylamine according to the method used in Interm diate 34 (a) in EP-A-494623.

Intermediate 18

N-Benzyl-N-methyl-2-(4-aminophenoxy)acetamide as an oil.

NMR includes signals at δ 4.8(s, 2H, O-CH_2-CO), 3.7(s, 2H, CH_2Ph), 2.8(s, 3H, N-CH_3). Prepared from Intermediate 17 according to the method used in Intermediate 35(a) in EP-A-494623.

Intermediate 19

4-[2-(N-Methylbenzylamino)ethoxy]aniline as a red oil. NMR includes signals at δ 3.9(t, 2H, 0-CH_2), 3.5(s, 2H, CH_2-Ph), 2.1(t, 2H, N-CH_2), 2.0(s, 3H, N-CH_3). Prepared from Intermediate 18 according to the method used in Intermediate 36(a) in EP-A-494623.

Intermediate 20

5-(3-Fluorobenzoyl)-2-methoxybenzoic acid

To a suspension of Intermediate 16 (4.3 g) in water (50 ml) was added potassium hydroxide (2.5 g) and the mixture was heated at reflux for 2 h. After cooling, the solution was acidified with 1N hydrochloric acid and the white precipitate was filtered off and dried to give the title compound (4 g) as a solid, mp : 200°.

Intermediate 21

Methyl 5-benzoyl-2-methoxybenzoate

Aluminium trichloride (16.2 g) and benzoyl chloride (7 ml) were added to 1,2-dichloroethane (100 ml) at room temperature. The mixture was cooled to - 5° and salicylic acid (8.3 g) was added portionwise and the mixture was heated to 60°. After 12 h at 60°, the mixture was cooled, poured into ice and acidified with 2N hydrochloric acid. Extraction with ethyl acetate and evaporation gave a white solid which was dissolved in dimethylsulphoxide (100 ml) and potassium carbonate (24 g) was added. After 1 h at room temperature, iodomethane (15 ml) was added and the mixture was heated at 40° for 3 h. Aft r cooling, the mixtur was poured in to ice and the precipitate was purified by chromatogaphy

on silica gel eluting with toluene/ethyl acetate (90/10) to give the title compound (11.5 g) as a solid, mp : 88°.

Intermediate 22

5-Benzoyl-2-methoxybenzoic acid

To a suspension of Intermediate 21 (7 g) in water (45 ml) was added potassium hydroxide (4.3 g) and the mixture was heated at reflux for 2 h. After cooling, the solution was acidified with 1N hydrochloric acid and the white precipitate was filtered off and dried to give the title compound (6.1 g) as a solid, mp : 150°.

Intermediate 23

Methyl 5-(3-methoxybenzoyl)-2-methoxybenzoate

Aluminium trichloride (9.4 g) and 3-methoxybenzoyl chloride (5 ml) were added to 1,2-dichloroethane (60 ml) at room temperature. The mixture was cooled to -5° and salicylic acid (4.8 g) was added portionwise and the mixture was heated to 40°. After 12 h at 40°, the mixture was cooled, poured into ice and acidified with 2N hydrochloric acid. Extraction with ethyl acetate and evaporation gave an oil which was dissolved in dimethylsulphoxide (50 ml) and potassium carbonate (20 g) was added. After 1 h at room temperature, iodomethane (10 ml) was added and the mixture was heated at 40° for 3 h. After cooling, the mixture was poured into ice and the oil was purified by chromatogaphy eluting with toluene/ethyl acetate (90/10) to give the title compound (4.1 g), as an yellow oil.

Intermediate 24

5-(3-Methoxybenzoyl)-2-methoxybenzoic acid

To a suspension of Intermediate 23 (3.5 g) in water (40 ml) was added potassium hydroxide (1.9 g) and the mixture was heated at reflux for 2 h. After cooling, the solution was acidified with 1N hydrochloric acid and the white precipitate was filtered off and dried to give the title compound (2.5 g) as a solid, mp : 132°.

Example 1

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]phenyl]-2-quinoxalinecarboxamide

A mixture of 2-quinoxalinecarboxylic acid (0.5g) and 1-hydroxybenzotriazole (0.39g) in DMF (20ml) was stirred at room temperature for 10min. 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]benzenamine (Intermediate 5(b) in EP-A-494623) (0.78g) was then added, followed by dicyclohexylcarbodiimide (0.59g) and the mixture was stirred at room temperature for 16h and then filtered. The filtrate was concentrated in vacuo, treated with dilute sodium hydroxide solution and extracted with methylene chloride. The combined, dried, organic extracts were evaporated and the residue was purified by column chromatography on silica gel eluting with methylene chloride/methanol (9:1) to give the title compound (0.62g) as a white solid, after crystallisation from methanol, mp = 155°.

Analysis Found : C, 71.41; H, 6.20; N, 11.62;

$C_{29}H_{30}N_4O_3$ ($0.25H_2O$) Requires : C, 71.51; H, 6.31; N, 11.50%.

The following compounds were prepared in a similar manner:

Example 2

N-[4-(3-(Methylveratrylamino)propyl)phenyl]-2-(4-methoxyphenyl)-4-quinolinecarboxamide

The coupling of 2-(4-methoxyphenyl)-4-quinolinecarboxylic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.9g) gave, after crystallisation from ethanol, the title compound as a solid (0.75g), mp = 105°.

Analysis Found : C, 75.24; H, 6.49; N, 7.20;

$C_{36}H_{37}N_3O_4$ Requires : C, 75.10; H, 6.48; N, 7.30%.

Example 3

N-[4-(2-(Methylveratrylamino)ethoxy)phenyl]-2-(3-methoxyphenyl)-4-quinolinecarboxamide

The coupling of 2-(3-methoxyphenyl)-4-quinolinecarboxylic acid (0.8g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzenemethanamine (Intermediate 36(b) in EP-A-494623) (0.78g) gave, after crystallisation from diisopropyl ether, the title compound as a solid (0.36g) mp = 97°.

Analysis Found : C, 72.55; H, 6.08; N, 7.23;

$C_{35}H_{35}N_3O_5$ Requires : C, 72.77; H, 6.11; N, 7.27%.

Example 4

N-[4-[2-[(4-Methoxybenzyl)methylamino]ethoxy]phenyl]-6-methyl-2-phenyl-4-quinolinecarboxamide

The coupling of 6-methyl-2-phenyl-4-quinolinecarboxylic acid (1.32g) with N-[2-(4-aminophenoxy)ethyl]-4-methoxy-N-methylbenzenemethanamine (Intermediate 36(f) in EP-A-494623) (1.2g) gave the title compound as an oil (0.6g) in the form of an oxalate (from isopropanol), mp = 180-182°.

Analysis Found : C, 67.66; H, 5.78; N, 6.91;

$C_{34}H_{33}N_3O_3$, $C_2H_2O_4$, Hb2O Requires : C, 67.59; H, 5.83; N, 6.57%.

Example 5

N-[4-[2-[(4-Methoxybenzyl)methylamino]ethoxy]phenyl]-6-methoxy-2-phenyl-4-quinolinecarboxamide

The coupling of 6-methoxy-2-phenyl-4-quinolinecarboxylic acid (0.84g) with N-[2-(4-aminophenoxy)ethyl]-4-methoxy-N-methylbenzenemethanamine (Intermediate 36(f) in EP-A-494623) (0.87g) gave after crystallisation from methanol, the title compound as a solid (0.25g), mp = 114 -115°.

Analysis Found : C, 73.94; H, 6.06; N, 7.81;

$C_{34}H_{33}N_3O_4$ Requires : C, 74.56; H, 6.07; N, 7.67%.

Example 6

N-[4-(4-(Methylveratrylamino)butyl)phenyl]-6-methoxy-2-phenyl-4-quinolinecarboxamide

The coupling of 6-methoxy-2-phenyl-4-quinolinecarboxylic acid (1.4g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenebutanamine (Intermediate 33(a) in EP-A-494623) (1.65g) gave, after crystallisation from ethanol, the title compound as a solid (0.38g), mp = 148°.

Analysis Found : C, 75.26; H, 6.69; N, 6.73;

$C_{37}H_{39}N_3O_4$ Requires : C, 75.74; H, 6.18; N, 7.16%.

Example 7

N-[4-(2-(Methylveratrylamino)ethyl)phenyl]-1-phenothiazinecarboxamide

The coupling of 1-phenothiazinecarboxylic acid* (0.63g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzeneethanamine (Intermediate 33(b) in EP-A-494623) (0.78g) gave the title compound as an oil (0.4g) in the form of a hydrochloride (from diethyl ether), mp = 144°.

Analysis Found : C,64.36; H,5.98; Cl,5.24; N,7.15; S, 5.60;

$C_{31}H_{31}N_3O_3S_1$, HCl, H_2ORequires : C,64.18; H,5.91; Cl,6.00; N,7.24; S, 5.53%.

* Brian D Palmer et al., J Med Chem 1988, 31, 707-712.

Example 8

N-[4-(2-(Methylveratrylamino)ethoxy)phenyl]-1-phenazin carboxamide

The coupling of 1-phenazinecarboxylic acid* (0.68g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dim thoxy)-N-methylbenzenemethanamine

(Intermediate 36(b) in EP-A-494623) (1g) gave, after crystallisation from ethanol, the title compound as a solid (0.55g), mp = 135°.

Analysis	Found : C, 71.30; H, 5.78; N, 10.47;
$C_{31}H_{30}N_4O_4$	Requires : C, 71.24; H, 5.78; N, 10.72%.

* Gordon W. Rewcastle et al., J Med Chem. 1987, 30, 843-851.

Example 9

N-[4-[2-[(4-Methoxybenzyl)methylamino]ethoxy]phenyl]-1-phenazinecarboxamide

The coupling of 1-phenazinecarboxylic acid (0.68g) with N-[2-(4-aminophenoxy)ethyl]-4-methoxy-N-methybenzenemethanamine (Intermediate 36 (f) in EP-A-494623) (1g) gave, after crystallisation from ethanol, the title compound as a solid (0.52g), mp = 134°.

Analysis	Found : C, 72.89; H, 5.76; N, 11.54;
$C_{30}H_{28}N_4O_3$	Requires : C, 73.15; H, 5.73; N, 11.37%.

Example 10

N-[4-(2-(Methylhomoveratrylamino)ethoxy)phenyl]-1-phenothiazine carboxamide

The coupling of 1-phenothiazinecarboxylic acid (0.73g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzeneethanamine (Intermediate 36(a) in EP-A-494623) (1.1g) gave, after crystallisation from ethanol, the title compound as a solid (0.45g), mp = 90°.

Analysis	Found : C,68.98; H,5.89; N,7.49; S,5.59;
$C_{32}H_{33}N_3O_4S_1$	Requires : C,69.16; H,5.98; N,7.56; S,5.77%.

Example 11

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]phenyl]-3-isoquinolinecarboxamide

The coupling of 3-isoquinolinecarboxylic acid (0.6g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolyl)propyl]benzenamine (Intermediate 5(b) in EP-A-494623) (1g) gave, after trituration in diethyl ether, the title compound (0.89g) as a solid, mp = 146°.

Analysis	Found : C, 73.87; H, 6.15; N, 8.60;
$C_{30}H_{31}N_3O_3$	Requires : C, 73.44; H, 6.57; N, 8.56%.

Example 12

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-6,7-dimethyl-2-quinoxalinecarboxamide

The coupling of 6,7-dimethyl-2-quinoxalinecarboxylic acid (0.45g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.68g) gave, after crystallisation from isopropanol, the title compound (0.26g) as a solid, mp = 100- 105°.

Analysis	Found : C, 70.82; H, 6.89; N, 10.23;
$C_{32}H_{36}N_4O_3$ (H_2O)	Requires : C, 70.82; H, 7.05; N, 10.32%.

Example 13

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-6(7)-methyl-2-quinoxalinecarboxamide

The coupling of 6(7)-methyl-2-quinoxalinecarboxylic acid* (0.5g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623) (0.89g) gave, after crystallisation from acetonitrile, the title compound (1g) as a solid, mp = 147°.

Analysis Found : C, 70.29; H, 6.33; N, 10.38;

$C_{31}H_{34}N_4O_4$ Requires : C, 70.70; H, 6.51; N, 10.64%.

*Chem, Abstracts 53,1358f.

Example 14

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]phenyl]-6(7)-methyl-2-quinoxalinecarboxamide

The coupling of 6(7)-methyl-2-quinoxalinecarboxylic acid (0.5g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]benzenamine (Intermediate 5(b) in EP-A-494623) (0.9g) gave, after crystallisation from isopropanol, the title compound (1.05g) as a solid, mp = 120-126°.

Analysis Found : C, 72.88; H, 6.89; N, 10.69;

$C_{31}H_{34}N_4O_3$ Requires : C, 72.92; H, 6.71; N, 10.97%.

Example 15

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-6(7)-methoxy-2-quinoxalinecarboxamide

The coupling of Intermediate 6 (0.54g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.9g) gave, after crystallisation from a 1:1 mixture of isopropanol and acetonitrile, the title compound (0.93g) as a solid, mp = 138°.

Analysis Found : C, 69.49; H, 6.41; N, 10.30;

$C_{31}H_{34}N_4O_4$ ($0.5H_2O$) Requires : C, 69.51; H, 6.59; N, 10.44%.

Example 16

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-6(7)-methoxy-2-quinoxalinecarboxamide

The coupling of Intermediate 6 (0.54g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623) (0.89g) gave, after crystallisation from a 1:1 mixture of isopropanol and acetonitrile, the title compound (0.9g) as a solid, mp = 166°.

Analysis Found : C, 67.24; H, 5.99; N, 10.48;

$C_{30}H_{32}N_4O_5$ ($0.5H_2O$) Requires : C, 67.02; H, 6.18; N, 10.42%.

Example 17

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]phenyl]-3-quinolinecarboxamide

The coupling of 3-quinolinecarboxylic acid (1g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]benzenamine (Intermediate 5(b) in EP-A-494623) (1.2g) gave, after crystallisation from isopropanol, the title compound (1.01g) as a solid, mp = 184-185°.

Analysis Found : C, 74.40; H, 6.50; N, 8.59;

$C_{30}H_{31}N_3O_3$ Requires : C, 74.82; H, 6.49; N, 8.73%.

Example 18

Hydrochloride salt of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-2-quinolinecarboxamide

The coupling of 2-quinolinecarboxylic acid (0.38g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.5g) gave, after crystallisation from isopropanol, the title compound (0.23g) as a solid, mp = 230-235°.

Analysis Found : C, 69.48; H, 6.45; N, 7.46;

$C_{31}H_{34}N_3O_3$ Requires : C, 69.98; H, 6.44; N, 7.90%.

Example 19

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]phenyl]-4-methoxy-2-quinolinecarboxamide

The coupling of 4-methoxy-2-quinolinecarboxylic acid (1g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]benzenamine (Intermediate 5(b) in EP-A-494623) (1g) gave, after crystallisation from isopropanol, the title compound (0.5g) as a solid, mp = 123-125°.

Analysis Found : C, 72.70; H, 6.58; N, 8.30;

$C_{31}H_{33}N_3O_4$ Requires : C, 72.78; H, 6.50; N, 8.21%.

Example 20

N-[4-[4-(Methylveratrylamino)butyl]phenyl]-2-quinoxalinecarboxamide

The coupling of 2-quinoxalinecarboxylic acid (0.5g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenebutanamine (Intermediate 33(a) in EP-A-494623) (0.94g) gave, after crystallisation from ethanol, the title compound (0.4g) as a solid, mp = 82-85°.

Analysis Found : C, 71.89; H, 6.73; N, 11.75;

$C_{29}H_{32}N_4O_3$ Requires : C, 71.88; H, 6.66; N, 11.56%.

Example 21

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-2-quinoxalinecarboxamide

The coupling of 2-quinoxalinecarboxylic acid (0.5g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamin (Intermediate 2(d) in EP-A-494623) (0.62g) gave, after trituration with diethyl ether, the title compound (0.4g) as a solid, mp = 144°.

Analysis Found : C, 72.33; H, 6.55;

$C_{30}H_{32}N_4O_3$ Requires : C, 72.56; H, 6.49%.

Example 22

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-2-quinoxalinecarboxamide

The coupling of 2-quinoxalinecarboxylic acid (0.5g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623 (1g) gave, after recrystallisation from ethanol, the title compound (0.78g) as a solid, mp = 170-173°.

Analysis Found : C, 69.35; H, 6.16; N, 11.27;

$C_{29}H_{30}N_4O_4$ Requires : C, 69.86; H, 6.06; N, 11.24%.

Example 23

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-3-methoxy-6,7-dimethyl-2-quinoxalinecarboxamide

The coupling of Intermediate 3(a) (0.6g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623 (0.8g) gave, after crystallisation from isopropanol, the title compound (0.47g) as a solid, mp = 158°.

Analysis Found : C, 67.32; H, 6.67; N, 9.80;

$C_{32}H_{36}N_4O_5$ ($0.5H_2O$) Requires : C, 67.94; H, 6.59; N, 9.90%.

Example 24

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]phenyl]-3-methoxy-6,7-dimethyl-2-quinoxalinecarboxamide

Th coupling of Intermediate 3(a) (0.6g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]benzenamine (Intermediate 5(b) in EP-A-

494623) (0.8g) gave, after crystallisation from isopropanol, the title compound (0.75g) as a solid, mp = 164-166°.

Analysis	Found : C, 67.32; H, 6.67; N, 9.80;
$C_{32}H_{36}N_4O_5$ (0.5H_2O)	Requires : C, 67.94; H, 6.54; N, 9.90%.

Example 25

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-3-methyl-2-quinoxalinecarboxamide

The coupling of 3-methyl-2-quinoxalinecarboxylic acid* (0.5g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.9g) gave, after crystallisation from a 1:1 mixture of isopropanol and acetonitrile, the title compound (0.9g) as a solid, mp = 146°.

Analysis	Found : C, 73.13; H, 6.76; N, 10.88;
$C_{31}H_{34}N_4O_3$	Requires : C, 72.92; H, 6.71; n, 10.97%.

* Chem Abstracts 46,8124c.

Example 26

N-[4-[3-(Methylveratrylamino)propyl]phenyl]-5-methoxyindole-2-carboxamide

The coupling of 5-methoxyindole-2-carboxylic acid (0.5g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.62g) gave, after crystallisation from isopropanol, the title compound (0.48g) as a solid, mp = 80°.

Analysis	Found : C, 70.79; H, 6.86; N, 8.02;
$C_{29}H_{33}N_3O_4$ (0.25H_2O)	Requires : C, 70.78; H, 6.86; N, 8.03%.

Example 27

N-[4-[3-(Methylveratrylamino)propyl]phenyl]-3-benzoyl-2-indolecarboxamide

The coupling of 3-benzoyl-2-indolecarboxylic acid (0.35g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.42g) gave, after crystallisation from ethanol, the title compound (0.30g) as a solid, mp = 156-161°.

Analysis Found : C, 74.25; H, 6.36; N, 7.05;

$C_{35}H_{35}N_3O_4$ ($0.25H_2O$) Requires : C, 74.24; H, 6.32; N, 7.42%.

Example 28

N-[4-[3-(Methylveratrylamino)propyl]phenyl]-1-naphtalenecarboxamide

The coupling of 1-naphthoic acid (0.3 g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.53 g) gave, after crystallisation from diisopropyl ether, the title compound (0.38 g) as a solid, mp : 113-117°.

Analysis Found : C,75.84; H,6.93; N,5.92;

$C_{30}H_{32}N_2O_3.0.4H_2O$ Requires : C,75.73; H,6.94; N,5.88%.

Example 29

Oxalate of N-[4-[3-methylveratrylamino]propyl]phenyl]-2-naphtalenecarboxamide

The coupling of 2-naphthoic acid (0.4 g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.73 g) gave the title compound (0.6 g) as a solid, mp : 203-207°.

Analysis Found : C,68.76; H,6.17; N,5.04;

$C_{30}H_{32}N_2O_3.C_2H_2O_4$ Requires : C,68.80; H,6.13; N,5.01%.

Example 30

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-2-naphtalenecarboxamide

The coupling of 2-naphthoic acid (0.6 g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.79 g) gave, after crystallisation from isopropanol, the title compound (0.5 g) as a solid, mp : 165-167°.

Analysis	Found : C,76.84; H,6.92; N,5.59;
$C_{32}H_{34}N_2O_3.0.3H_2O$	Requires : C,76.86; H,6.97; N,5.60%.

Example 31

N-[4-[2-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-2isoquinolinyl)ethyl]phenyl]-2-naphtalenecarboxamide

The coupling of 2-naphthoic acid (0.47 g) with 4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]benzenamine (Intermediate 2(c) in EP-A-494623) (0.82 g) gave, after crystallisation from isopropanol, the title compound (0.83 g) as a solid, mp : 162-165°.

Analysis	Found : C,77.28; H,6.50; N,5.91;
$C_{30}H_{30}N_2O_3$	Requires : C,77.23; H,6.48; N,6.00%.

Example 32

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-2-naphtalenecarboxamide

The coupling of 2-naphthoic acid (0.3 g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623) (0.58 g) gave, after crystallisation from acetonitrile, the title compound (0.2 g) as a solid, mp : 189-190°.

Analysis Found : C,74.97; H,6.53; N,5.54;

$C_{31}H_{32}N_2O_4$ Requires : C,74.98; H,6.50; N,5.64%.

Example 33

N-[4-[3-(Methylveratrylamino)propoxy]phenyl]-2-naphtalenecarboxamide

The coupling of 2-naphthoic acid (0.4 g) with N-[3-(4-aminophenoxy)propyl]-3,4-dimethoxy-N-methylbenzenemethanamine (Intermediate 38(c) in EP-A-494623) (0.76 g) gave, after crystallisation from acetonitrile, the title compound (0.45 g) as a solid, mp : 131-133°.

Analysis Found : C,74.22; H,6.75; N,5.78;

$C_{30}H_{32}N_2O_4$ Requires : C,74.36; H,6.66; N,5.78%.

Example 34

Oxalate of N-[4-[3-methylveratrylamino]propyl]phenyl]-1-isoquinolinecarboxamide

The coupling of 1-isoquinolinecarboxylic acid (0.35 g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(c) in EP-A-494623) (0.53 g) gave the title compound (0.3 g) as a solid, mp : 183-187°.

Analysis Found : C,66.65; H,6.00; N,7.40;

$C_{29}H_{31}N_3O_3.C_2H_2O_4$ Requires : C,66.53; H,5.94; N,7.51%.

Example 35

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-1-isoquinolinecarboxamide

The coupling of 1-isoquinolinecarboxylic acid (0.35 g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benz namine (Intermediate 2(a) in EP-A-494623) (0.58 g) gave, after crystallisation from isopropanol, th title compound (0.6 g) as a solid, mp : 160°.

Analysis Found : C,72.61; H,6.39; N,8.43;

$C_{30}H_{31}N_3O_4$ Requires : C,72.41; H,6.28; N,8.44%.

Example 36

Oxalate of N-[4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]phenyl]-1-isoquinolinecarboxamide

The coupling of 1-isoquinolinecarboxylic acid (0.35 g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]benzenamine (Intermediate 5(b) in EP-A-494623) (0.55 g) gave the title compound (0.5 g) as a solid, mp : 206-209°.

Analysis Found : C,66.56; H,5.87; N,7.30;

$C_{30}H_{31}N_3O_3$ $C_2H_2O_4$ $0.3H_2O$ Requires :C,66.60; H,5.87; N,7.28%.

Example 37

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-3-(2-methoxybenzoyl)benzamide

The coupling of Intermediate 8 (0.56 g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.67 g) gave the title compound (0.31 g) as an amorphous solid, mp = 78°.

Analysis Found : C,71.35; H,6.68; N,4.82;.

$C_{36}H_{38}N_2O_5$ $1.5H_2O$ Requires : C,71.38; H,6.82; N,4.62%

Example 38

Fumarate of N-[4-[3-methylveratrylamino]propyl]phenyl]-2-indolecarboxamide

The coupling of 2-indolecarboxylic acid (0.3 g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.58 g) gave the title compound (0.3 g) as a solid, mp = 196°.

Analysis Found : C,69.79; H,6.36; N,8.21;

$C_{28}H_{31}N_3O_3\ C_4H_4O_4$ Requires : C,69.88; H,6.45; N,8.15%.

Example 39

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butoxy]phenyl]-6(7)-methyl-2-quinoxalinecarboxamide

The coupling of 6(7)-methyl-2-quinoxalinecarboxylic acid (0.5g) with Intermediate 11(b) (0.94g) gave, after crystallisation from a 1:1 mixture of isopropanol and acetonitrile, the title compound (1.09g) as a solid, mp = 142-148°.

Analysis Found : C, 70.86; H, 6.49; N, 10.40;

$C_{31}H_{34}N_4O_4$ Requires : C, 70.70; H, 6.51; N, 10.64%.

Example 40

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butoxy]phenyl]-1-isoquinolinecarboxamide

The coupling of 1-isoquinolinecarboxylic acid (0.5g) with Intermediate 11(b) (0.89g) gave, after crystallisation from methanol, the title compound (0.6g) as a solid, mp = 122-123°.

Analysis Found : C, 72.73; H, 6.62; N, 8.12;

$C_{31}H_{33}N_3O_4$ Requires : C, 72.78; H, 6.50; N, 8.21%.

Example 41

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butoxy]phenyl]-2-quinoxalinecarboxamide

The coupling of 2-quinoxalinecarboxylic acid (0.5g) with Intermediate 11(b) (0.89g) gave, after crystallisation from acetonitrile, the title compound (0.97g) as a solid, mp = 141°.

Analysis Found : C, 69.62; H, 6.29; N, 10.93;

$C_{30}H_{32}N_4O_4$ ($0.3H_2O$) Requires : C, 69.55; H, 6.34; N, 10.81%.

Example 42

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butoxy]phenyl]-3-ethoxy-2-quinoxalinecarboxamide

The coupling of 3-ethoxy-2-quinoxalinecarboxylic acid (0.5g) with Intermediate 11(b) (0.63g) gave, after crystallisation from ethanol, the title compound (0.48g) as a solid, mp = 182°.

Analysis Found : C, 72.08; H, 4.51; N, 16.86;

$C_{18}H_{11}N_3O$ Requires : C, 72.28; H, 4.45; N, 16.86%.

Example 43

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butoxy]phenyl]-4-[2-(4-chlorophenyl)-3-trifluoromethylpyrazole]carboxamide

The coupling of 2-(4-chlorophenyl)-3-trifluoromethylpyrazole-4-carboxlic acid (1g) with Intermediate 11(b) (1.3g) gave the title compound (1.8g), mp = 153°.

Analysis Found : C,60.87; H,5.11; N,8.77;

$C_{32}H_{32}ClF_3N_4O_4$ Requires: C,61.10; H,5.13; N,8.91%.

Example 44

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butoxy]phenyl]-5-[4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]carboxamide

The coupling of 4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole-5-carboxylic acid (1g) with Intermediate 11(b) (1g) gave, after crystallisation from methanol/ethanol (1:1), the title compound (0.7g), mp = 160-180°.

Analysis Found : C,62,95; H,5.33; F,9.06;, N,6.52;

$C_{33}H_{34}F_3N_3O_4S$ Requires : C,63.35; H,5.48; F,9.11; N,6.72%.

Example 45

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-2-[5-(2-pyridyl)thiophene]carboxamide

The coupling of 5-(2-pyridyl)thiophene-2-carboxylic acid (1g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623) (1.3g) gave, after crystallisation from methanol, the title compound (1.5g), mp = 196°.

Analysis Found : C,67.96; H,5.88; N,7.86;

$C_{30}H_{31}N_3O_4S$ Requires : C,68.03; H,5.90; N,7.93%.

Example 46

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-4-[2-(3-pyridyl)thiazole]carboxamide

The coupling of 2-(3-pyridyl)thiazole-4-carboxylic acid (1g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623) gave, after crystallisation from isopropanol/methanol, the title compound (1.2g), mp = 125°.

Analysis Found : C,65,30; H,5.11; N, 10.32;

$C_{29}H_{30}N_4O_4S$ Requires : C,65.64; H,5.70; N,10.56%

Example 47

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-5-(4-methyl-2-phenyl-1,2,3-triazole)carboxamide

The coupling of 4-methyl-2-phenyl-1,2,3-triazole-5-carboxylic acid (1g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benz namin

(Intermediate 2(a) in EP-A-494623) (1.6g) gave, after crystallisation from methanol/pyridine (5:1) the title compound (1.6g), mp = 146°.

Analysis Found : C,67.28; H,6.10; N,13.20;

$C_{30}H_{33}N_5O_4$ ($0.5H_2O$) Requires : C,67.14; H,6.38; N,13.05%.

Example 48

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-2-hydroxypropoxy]phenyl]-2-quinoxalinecarboxamide

The coupling of 2-quinoxalinecarboxylic acid (0.5g) with Intermediate 13 (1g) gave the title compound (0.9g) as a solid, mp = 158-160°.

Analysis Found : C,65.68; H,5.99; N,10.23;

$C_{29}H_{30}N_4O_5$ (1 H_2O) Requires : C,65.40; H,6.05:N,10.52%.

Example 49

N-[4-(2-(Methylveratrylamino)ethyl)phenyl]-2-(4-methoxyphenyl)-4-quinolinecarboxamide

The coupling of 2-(4-methoxyphenyl)-4-quinolinecarboxylic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzeneethanamine (Intermediate 33(b) in EP-A-494623) (0.86g) gave, after crystallisation from ethanol, the title compound as a solid (0.33g), mp = 114°.

Analysis Found : C, 74.72; H, 6.29; N, 7.29;

$C_{35}H_{35}N_3O_4$ Requires : C, 74.84; H, 6.28; N, 7.48%.

Example 50

N-[4-(3-(Methylveratrylamino)propyl)phenyl]-2-(3-methoxyphenyl)-4-quinolinecarboxamide

The coupling of 2-(3-methoxyphenyl)-4-quinolinecarboxylic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine

(Intermediate 33(f) in EP-A-494623) (0.9g) gave, after crystallisation from isopropanol, the title compound as a solid (0.51g), mp = 110°.

Analysis Found : C, 75.10; H, 6.52; N, 7.26;

$C_{36}H_{37}N_3O_4$ Requires : C, 75.10; H, 6.48; N, 7.30%.

Example 51

N-[4-[2-(Methylveratrylamino)ethyl]phenyl]-9-oxo-4-thioxanthenecarboxamide

A mixture of 9-oxo-4-thioxanthenecarboxylic acid* (0.8g) and 1-hydroxybenzotriazole (0.42g) in DMF (20 ml) was stirred at room temperature for 10 min. 4-Amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenemethanamine (Intermediate 33(b) in EP-A-494623) (0.94g) in DMF (20 ml) was then added, followed by dicyclohexylcarbodiimide (0.64g) and the mixture was stirred at room temperature for 16 h and then filtered. The filtrate was concentrated in vacuo, treated with dilute sodium hydroxide solution and extracted with dichloromethane. The combined dried organic extracts were evaporated to leave an oil which was purified by column chromatography eluting with dichloromethane : methanol (95:5). The resulting solid was recrystallised from acetonitrile and filtered off to give the title compound as a solid (0.26g),mp = 180°.

Analysis Found : C, 71.02; H, 5.59; N, 5.18; S, 5.78;

$C_{32}H_{30}N_2O_4S_1$ Requires : C, 71.35; H, 5.61; N, 5.20; S, 5.95%.

*Chem.Abstracts 99,5518d.

The following examples were prepared in a similar manner:

Example 52

N-[4-(3-(Methylveratrylamino)propyl)phenyl]-5-methoxy-9-oxo-4-thioxanthenecarboxamide

The coupling of 5-methoxy-9-oxo-4-thioxanthenecarboxylic acid* (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine

(Intermediate 33(f) in EP-A-494623) (0.88g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.12g), mp = 144 - 146°.

Analysis Found : C, 69.49; H, 5.86; N, 4.75; S, 5.33;

$C_{34}H_{34}N_2O_5S_1$ Requires : C, 70.08; H, 5.88; N, 4.81; S, 5.50%.

*prepared from 2-(methoxyphenylthio)isophtalic acid** in sulphuric acid, mp>200°, IR includes peaks at 1660cm^{-1}(CO) and 1700cm^{-1}(CO_2H), by a method analogous to that described in Chem.Abstracts 99, 5518d.

**prepared from 2-iodorsophtalic acid and 2-methoxythiophenol, mp = 208°, IR includes a broad band at 1700-1720cm^{-1} (CO_2H), by a method analogous to that described in Chem. Abstracts 99, 5518d.

Example 53

N-[4-(2-(Methylveratrylamino)ethyl)phenyl]-5-methoxy-9-oxo-4-thioxanthenecarboxamide

The coupling of 5-methoxy-9-oxo-4-thioxanthenecarboxylic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzeneethanamine (Intermediate 33(b) in EP-A-494623) (0.8g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.1g) mp = 151°.

Analysis Found : C, 67.98; H, 5.66; N, 4.79; S, 5.29;

$C_{33}H_{32}N_2O_5S_1,H2O$ Requires : C, 67.55; H, 5.84; N, 4.77; S, 5.46%.

Example 54

N-[4-(3-(Methylveratrylamino)propoxy)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzeneethanamine (Intermediate 33(b) in EP-A-494623) (1g) gave, after crystallisation from ethanol, the titl compound as a solid (0.47g), mp = 184°.

Analysis Found : C, 69.67; H, 5.68; N, 4.93; S, 5.52;

$C_{33}H_{32}N_2O_5S_1$ Requires : C, 69.69; H, 5.67; N, 4.93; S, 5.64%.

Example 55

N-[4-(2-(Methylveratrylamino)ethyl)phenyl]-7-fluoro-9-oxo-4-thioxanthenecarboxamide

The coupling of 7-fluoro-9-oxo-4-thioxanthenecarboxylic acid* (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzeneethanamine (Intermediate 33(b) in EP-A-494623) (0.87g) gave, after crystallisation from ethanol, the title compound as a solid (0.3g), mp = 205°.

Analysis Found : C, 68.99; H, 5.23; F, 3.31; N, 4.99; S, 5.58;

$C_{32}H_{29}F_1N_2O_4S_1$ Requires : C, 69.04; H, 5.25; F, 3.41; N, 5.03;

S, 5.76%.

*prepared from 2-(4-fluorophenylthio)isophtalic acid** in sulphuric acid, mp>200°, IR includes peaks at 1660cm^{-1} (CO) and 1700cm^{-1}(CO_2H), by a method analogous to that described in Chem. Abstracts 99, 5518d.

**prepared from 2-iodoisophtalic acid and 4-fluorothiophenol, mp = 204-205°, IR includes a large band at 1700cm^{-1}(CO_2H).

Example 56

N-[4-(3-(Methylveratrylamino)propyl)phenyl]-7-fluoro-9-oxo-4-thioxanthenecarboxamide

The coupling of 7-fluoro-9-oxo-4-thioxanthenecarboxylic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.9g) gave, after crystallisation from acetonitrile, th titl compound as a solid (0.3g) mp = 160°.

Analysis Found : C, 69.24; H, 5.46; F, 3.20; N, 4.85;

S, 5.49;

$C_{33}H_{31}F_1N_2O_4S_1$ Requires : C, 69.45; H, 5.48; F, 3.33; N, 4.91;

S, 5.62%.

Example 57

N-[4-(4-(Methylveratrylamino)butyl)phenyl]-7-fluoro-9-oxo-4-thioxanthenecarboxamide

The coupling of 7-fluoro-9-oxo-4-thioxanthenecarboxylic acid (0.4g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenebutanamine (Intermediate 33(a) in EP-A-494623) (0.48g) gave, after crystallisation from ethanol the title compound as a solid (0.076g), mp = 168°.

Analysis Found : C, 69.80; H, 5.77; F, 3.24; N, 4.66;

S, 5.42;

$C_{34}H_{33}F_1N_2O_4S_1$ Requires : C, 69.84; H, 5.69; F, 3.25; N, 4.79;

S, 5.48%.

Example 58

N-[4-(3-(Methylveratrylamino)propylthio)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with N-[3-[(4-aminophenyl)thio]propyl]-3,4-dimethoxy-N-methylbenzenemethanamine (Intermediate 38(d) in EP-A-494623) (1g) gave, after crystallisation from ethanol, the title compound as a solid (0.1g), mp = 148°.

Analysis Found : C, 67.73; H, 5.35; N, 4.71; S, 10.85;

$C_{33}H_{32}N_2O_4S_2$ Requires : C, 67.78; H, 5.52; N, 4.79; S, 10.96%.

Example 59

N-[4-(Methylveratrylamino)methyl)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with Intermediate 11(d) (0.9g) gave, after crystallisation from ethanol, the title compound as a solid (0.1g), mp = 166°.

Analysis	Found :	C, 70.85; H, 5.38; N, 5.50; S, 5.90;
$C_{31}H_{28}N_2O_4S_1$	Requires :	C, 70.97; H, 5.38; N, 5.34; S, 6.11%.

Example 60

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623) (1.14g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.35g), mp = 210°.

Analysis	Found :	C, 70.29; H, 5.51; N, 4.89; S, 5.52;
$C_{34}H_{32}N_2O_5S_1$	Requires :	C, 70.32; H, 5.55; N, 4.83; S, 5.52%.

Example 61

N-[4-[2-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-5-methoxy-9-oxo-4-thioxanthenecarboxamide

The coupling of 5-methoxy-9-oxo-4-thioxanthenecarboxylic acid (3g) with 4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]benzenamine (Intermediate 2(c) in EP-A-494623) (3g) gave, after crystallisation from methanol, the title compound as a solid (1.38g), mp = 218 - 219°.

NMR includes signals at δ 2.8(4H,m,N-$(CH_2)_2$-Ph);

3.7(6H,s,2OCH_3); 3.8(3H,s,OCH_3).

Example 62

N-[4-(2-(Methylhomoveratrylamino)ethoxy)phenyl]-9-oxo-4-xanthenecarboxamide

The coupling of 9-oxo-4-xanthenecarboxylic acid (0.33g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzeneethanamine (Intermediate 36(a) in EP-A-494623) (0.45g) gave, after crystallisation from ethanol, the title compound as a solid (0.15g), mp = 152°.

Analysis	Found : C, 71.54; H, 5.85; N, 5.07;
$C_{33}H_{32}N_2O_6$	Requires : C, 71.72; H, 5.84; N, 5.07%.

Example 63

N-[4-(2-(Methylhomoveratrylamino)ethoxy)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzeneethanamine (Intermediate 36(a) in EP-A-494623) (1g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.35g), mp = 168°.

Analysis	Found : C, 69.71; H, 5.67; N, 4.91; S, 5.50;
$C_{33}H_{32}N_2O_5S_1$	Requires : C, 69.69; H, 5.67; N, 4.93; S, 5.64%.

Example 64

N-[4-(2-(Methylveratrylamino)ethoxy)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (1g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzenemethanamine (Intermediate 36(b) in EP-A-494623) (1.23g) gave, after crystallisation from acetonitril , the titl compound as a solid (0.2g), mp = 188°.

Analysis Found : C, 68.89; H, 5.75; N, 5.50; S, 5.46;

$C_{32}H_{30}N_2O_5S_1$ Requires : C, 69.29; H, 5.45; N, 5.05; S, 5.78%.

Example 65

N-[4-(3-(Methylhomoveratrylamino)propoxy)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with N-[3-(4-aminophenoxy)propyl]-3,4-dimethoxy-N-methylbenzeneethanamine (Intermediate 38(a) in EP-A-494623) (1g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.6g), mp = 174°.

Analysis Found : C, 69.70; H, 5.89; N, 4.70; S, 5.39;

$C_{34}H_{34}N_2O_5S_1$ Requires : C, 70.08; H, 5.88; N, 4.81; S, 5.50%.

Example 66

N-[4-(4-(Methylveratrylamino)butyl)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.77g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenebutanamine (Intermediate 33(a) in EP-A-494623) (0.98g) gave, after crystallisation from ethanol, the title compound as a solid (0.27g), mp = 156°.

Analysis Found : C, 71.82; H, 6.00; N, 5.06; S, 5.63;

$C_{34}H_{34}N_2O_4S_1$ Requires : C, 72.05; H, 6.05; N, 4.94; S, 5.66%.

Example 67

N-[4-(4-(Methylhomoveratrylamino)butyl)phenyl]-7-fluoro-9-oxo-4-thioxanthenecarboxamide

The coupling of 7-fluoro-9-oxo-4-thioxanthenecarboxylic acid (1g) with 4-amino-N-[2-(3,4-dimethoxyphenyl)ethyl]-N-methylbenzenebutanamine (Intermediate 33(c) in EP-A-494623) (1.25g) gave, after crystallisation from ethanol, the title compound as a solid (0.95g), mp = 145°.

Analysis Found : C, 69.87; H, 5.79; F, 2.95; N, 4.30;

S, 5.35;

$C_{35}H_{35}F_1N_2O_4S_1$ Requires : C, 70.21; H, 5.89; F, 3.17; N, 4.68;

S, 5.35%.

Example 68

N-[4-(2-(Methylhomoveratrylamino)ethoxy)phenyl]-7-fluoro-9-oxo-4-thioxanthenecarboxamide

The coupling of 7-fluoro-9-oxo-4-thioxanthenecarboxylic acid (1g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzeneethanamine (Intermediate 36(a) in EP-A-494623) (1.2g) gave, after crystallisation from ethanol, the title compound as a solid (0.72g), mp = 145°.

Analysis Found : C, 67.42; H, 5.26; F, 2.92; N, 4.92;

S, 5.85;

$C_{33}H_{31}F_1N_2O_5S_1$ Requires : C, 67.56; H, 5.33; F, 3.24; N, 4.77;

S, 5.46%.

Example 69

N-[4-(2-(Methyveratrylamino)ethoxy)phenyl]-9-oxo-4-xanthenecarboxamide

The coupling of 9-oxo-4-xanthenecarboxylic acid (0.6g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzenemethanamine (Intermediate 36(b) in EP-A-494623) (0.79g) gave, after crystallisation from ethanol, the title compound as a solid (0.21g), mp = 110°.

Analysis Found : C, 71.17; H, 5.59; N, 5.29;

$C_{32}H_{30}N_2O_6$ Requires : C, 71.36; H, 5.62; N, 5.20%.

Example 70

N-[4-(2-(Methylhomoveratrylamino)ethyl)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with 4-amino-N-[2-(3,4-dimethoxyphenyl)ethyl]-N-methylbenzeneethanamine (Intermediate 33(e) in EP-A-494623) (1g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.43g), mp = 154°.

Analysis	Found : C, 71.83; H, 5.92; N, 5.08; S, 5.89;
$C_{33}H_{32}N_2O_4S_1$	Requires : C, 71.71; H, 5.84; N, 5.07; S, 5.80%.

Example 71

N-[4-(4-(Methylhomoveratrylamino)butyl)phenyl]-9-oxo-4-xanthenecarboxamide

The coupling of 9-oxo-4-xanthenecarboxylic acid (0.3g) with 4-amino-N-[2-(3,4-dimethoxyphenyl)ethyl]-N-methylbenzenebutanamine (Intermediate 33(c) in EP-A-494623) (0.42g) gave, after crystallisation from ethanol, the title compound as a solid (0.09g), mp = 102°.

Analysis	Found : C, 73.58; H, 6.36; N, 5.07;
$C_{35}H_{36}N_2O_5$	Requires : C, 74.44; H, 6.43; N, 4.96%.

Example 72

N-[4-(3-(Methylhomoveratrylamino)propoxy)phenyl]-9-oxo-4-xanthenecarboxamide

The coupling of 9-oxo-4-xanthenecarboxylic acid (0.6g) with N-[3-(4-aminophenoxy)propyl]-3,4-dimethoxy-N-methylbenzeneethanamine Intermediate 38(a) in EP-A-494623) (1.04g) gave, after crystallisation from ethanol, the title compound as a solid (0.26g), mp = 126°.

Analysis Found : C, 71.27; H, 6.06; N, 4.84;

$C_{34}H_{34}N_2O_6$ Requires : C, 72.07; H, 6.05; N, 4.94%.

Example 73

N-[4-[4-[(4-Methylthiobenzyl)methylamino]butyl]phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with 4-amino-N-[[4-(methylthio)phenyl]methyl]-N-methylbenzenebutanamine (Intermediate 33(j) in EP-A-494623) (1g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.39g), mp = 167°.

Analysis Found : C, 71.47; H, 5.78; N, 5.13; S, 11.50;

$C_{33}H_{32}N_2O_2S_2$ Requires : C, 71.70; H, 5.84; N, 5.07; S, 11.60%.

Example 74

N-[4-[3-[(4-Methoxybenzyl)methylamino]propyl]phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.77g) with 4-amino-N-[(4-methoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(g) in EP-A-494623) (0.85g) gave, after crystallisation from ethanol, the title compound as a solid (0.34g), mp = 170°.

Analysis Found : C, 73.22; H, 5.84; N, 5.35; S, 5.89;

$C_{32}H_{30}N_2O_3S_1$ Requires : C, 73.53; H, 5.78; N, 5.36; S, 6.13%.

Example 75

N-[4-(3-(Methylhomoveratrylamino)propyl)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.8g) with 4-amino-N-[2-(3,4-dimethoxyph nyl)ethyl]-N-methylbenzenepropanamine (Intermediate 33(d)

in EP-A-494623) (1g) gave, after crystallisation from acetonitrile, the title compound as a solid (0.35g), mp = 143°.

Analysis Found : C, 72.10; H, 5.91; N, 4.70; S, 5.48;

$C_{34}H_{34}N_2O_4S_1$ Requires : C, 72.06; H, 6.05; N, 4.94; S, 5.66%.

Example 76

N-[4-[2-[(4-Methoxyphenethyl)methylamino]ethyl]phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.4g) with 4-amino-N-[2-(4-methoxyphenyl)ethyl]-N-methylbenzeneethanamine (Intermediate 33(k) in EP-A-494623) (0.44g) gave, after crystallisation from ethanol, the title compound as a solid (0.13g), mp = 163°.

Analysis Found : C, 72.49; H, 5.80; N, 5.35; S, 5.97;

$C_{32}H_{30}N_2O_3S_1$ Requires : C, 73.53; H, 5.79; N, 5.36; S, 6.13%.

Example 77

N-[4-(5-(Methylveratrylamino)pentyl)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.4g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepentanamine (Intermediate 33(l) in EP-A-494623) (0.53g) gave, after crystallisation from ethanol, the title compound as a solid (0.2g), mp = 166°.

Analysis Found : C, 72.31; H, 6.22; N, 4.85; S, 5.39;

$C_{35}H_{36}N_2O_4S_1$ Requires : C, 72.38; H, 6.25; N, 4.82; S, 5.52%.

Example 78

N-[4-(3-(Methylveratrylamino)propyl)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (3g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenz nepropanamin (Intermediat 33(f) in

EP-A-494623) (3.7g) gave, after crystallisation from ethanol, the title compound as a solid (2.5g), mp = 150°.

Analysis Found : C, 71.70; H, 5.88; N, 5.06; S, 5.72;

$C_{33}H_{32}N_2O_4S_1$ Requires : C, 71.71; H, 5.84; N, 5.07; S, 5.80%.

Example 79

N-[4-[3-(Methylveratrylamino)propyl]phenyl]-9-fluorenone-4-carboxamide

The coupling of 9-fluorenone-4-carboxylic acid (0.5g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.63g) gave, after crystallisation from ethanol, the title compound (0.75g) as a solid, mp = 50 - 75°.

Analysis Found : C, 75.12; H, 6.38; N, 5.23;

$C_{33}H_{32}N_2O_4$ ($0.4H_2O$) Requires : C, 75.09; H, 6.26; N, 5.23%.

Example 80

Fumarate of N-[4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propylthio]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.5 g) with 4-[[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propyl]thio]benzenamine (Intermediate 2(b) in EP-A-494623) (0.79 g) gave the title compound (0.4 g) as a solid, mp : 192°.

Analysis Found : C,66.94; H,5.68; N,4.07;

$C_{34}H_{34}N_2O_4S.C_4H_4O$ Requires : C,66.85; H,5.61; N,4.10%.

Example 81

Oxalate of N-[4-[3-(methylveratrylamino)propoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.8g) with N-[3-(4-aminophenoxy)propyl]-3,4-dimethoxy-N-methylbenzenemethanamine

(Intermediate 38(c) in EP-A-494623) (1.17g) gave the title compound (1.2g) as a solid, mp : 168°.

Analysis Found : C,66.92; H,5.79; N,4.42;

$C_{33}H_{34}N_2O_5.C_2H_2O_4$ Requires : C,66.87; H,5.77; N,4.46%.

Example 82

Fumarate of N-[4-[4-(methylveratrylamino)]butyl]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenebutanamine (Intermediate 33(a) in EP-A-494623) (1.16 g) gave the title compound (1.2g) as a solid, mp : 182°.

Analysis Found : C,70.06; H,6.19; N,4.22;

$C_{34}H_{36}N_2O_4.C_4H_4O_4$ Requires : C,69.92; H,6.18; N,4.29%.

Example 83

N-[4-[2-(Methylveratrylamino)ethyl]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.22g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzeneethanamine (Intermediate 33(b) in EP-A-494623) (0.3g) gave, after crystallisation from diisopropyl ether, the title compound (0.28g) as a solid, mp : 130°.

Analysis Found : C,75.19; H,6.37; N,5.50;

$C_{32}H_{32}N_2O_4$ Requires : C,75.57; H,6.34; N,5.51%.

Example 84

Fumarate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-3-benzoylbenzamide

The coupling of 3-benz ylbenzoic acid (0.8g) with 4-[4-(1,2,3,4-t trahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (1.2g) gav the titl compound (0.3g) as a solid, mp : 198°.

Analysis Found : C,70.36; H,6.03; N,4.08;

$C_{35}H_{36}N_2O_4.C_4H_4O_4$ Requires : C,70.46; H,6.06; N,4.21%.

Example 85

N-[4-[3-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (1g) with 4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]benzenamine (Intermediate 2(a) in EP-A-494623) (1.5g) gave, after crystallisation from isopropanol, the title compound (1.3g) as a solid, mp : >260°.

Analysis Found : C,74.12; H,6.18; N,5.16;

$C_{34}H_{34}N_2O_5$ Requires : C,74.15; H,6.22; N,5.08%.

Example 86

Oxalate of N-[2-methoxy-4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.6g) with Intermediate 11(a) (0.98g) gave the title compound (1g) as a solid, mp : 158°.

Analysis Found : C,66.29; H,5.72; N,4.10;

$C_{35}H_{36}N_2O_6.C_2H_2O_4$ Requires : C,66.26; H,5.71; N,4.18%.

Example 87

Fumarate of N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.6g) with 4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]benzenamine (Intermediate 2(c) in EP-A-494623) (0.82g) gave th titl compound (1g) as a solid, mp : 134°.

Analysis Found : C,70.87; H,5.84; N,4.33;

$C_{33}H_{32}N_2O_4.1/2\ C_4H_4O_4.1.5\ H_2O$ Requires : C,70.98; H,6.04; N,4.73%.

Example 88

Oxalate of N-[2-methyl-4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.86g) with 2-methyl-4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]benzenamine (Intermediate 16(c) in EP-A-494623) (1.25g) gave the title compound (0.6g) as a solid, mp : 230°.

Analysis Found : C,72.19; H,6.06; N,4.54;

$C_{34}H_{34}N_2O_4.1/2\ C_2H_2O_4$ Requires : C,72.51; H,6.08; N,4.83%.

Example 89

Fumarate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-3-(3-methoxybenzoyl)benzamide

The coupling of Intermediate 14 (0.5g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.63g) gave, the title compound (0.7g) as a solid, mp : 188°.

Analysis Found : C,69.13; H,6.04; N,4.13;

$C_{36}H_{38}N_2O_5.C_4H_4O_4$ Requires : C,69.15; H,6.09; N,4.03%.

Example 90

Fumarate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-3-(4-fluorobenzoyl)benzamide

The coupling of Intermediate 15 (0.46g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.64g) gave, th title compound (0.25g) as a solid, mp : 176°.

Analysis Found : C,68.51; H,5.85; F,2.86; N,4.31;

$C_{35}H_{35}FN_2O_4.C_4H_4O_4$ Requires : C,68.61; H,5.76; F,2.78; N,4.10%.

Example 91

Fumarate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-3-(4-methoxybenzoyl)benzamide

The coupling of 3-(4-methoxybenzoyl)benzoic acid* (0.4g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.53g) gave the title compound (0.55g) as a solid, mp : 178°.

Analysis Found : C,68.85; H,6.01; N,4.12;

$C_{36}H_{38}N_2O_5.C_4H_4O_4$ Requires : C,69.15; H,6.09; N,4.03%.

* A.I. Meyers et al., J.Amer. Chem. Soc., 91 (21), 5886-87 (1969).

Example 92

Oxalate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-5-(3-fluorobenzoyl)-2-methoxy-benzamide

The coupling of Intermediate 20 (0.5g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.62g) gave, the title compound (0.6g) as a solid, mp : 112°.

Analysis Found : C,66.23; H,5.73; F,2.85; N,4.02;

$C_{36}H_{37}FN_2O_5.C_2H_2O_4$ Requires : C,66.46; H,5.72; F,2.77; N,4.08%.

Example 93

Oxalate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-5-benzoyl-2-methoxybenzamide

The coupling of Intermediate 22 (0.5g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.66g) gave the titl compound (1g) as a solid, mp : 202°.

Analysis Found : C,68.16; H,6.04; N,4.13;

$C_{36}H_{38}N_2O_5.C_2H_2O_4$ Requires : C,68.25; H,6.03; N,4.19%.

Example 94

Oxalate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-5-(3-methoxybenzoyl)-2-methoxybenzamide

The coupling of Intermediate 24 (0.5g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.59g) gave the title compound (0.8 g) as a solid, mp : 116°.

Analysis Found : C,65.24; H,6.18; N,3.81;

$C_{37}H_{40}N_2O_6.C_2H_2O_4.1H_2O$ Requires : C,65.35; H,6.18; N,3.90%.

Example 95

Oxalate of N-[4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-5-(3-methylbenzoyl)-2-methoxybenzamide

The coupling of 5-(3-methylbenzoyl)-2-methoxybenzoic acid* (0.42g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.53g) gave the title compound (0.45g) as a solid, mp : 114°.

Analysis Found : C,67.56; H,6.34; N,3.89;

$C_{37}H_{40}N_2O_5.C_2H_2O_4.1/2H_2O$ Requires : C,67.71; H,6.26; N,4.04%.

* Fujii Yasao et al., Nippon Noyaku Gakkaishi, 4 (4), 511-514 (1979).

Example 96

Fumarate of N-[2-methyl-4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (1g) with Intermediate 11(c) (1.4g) gave the title compound (0.9g) as a solid, mp = 94°.

Analysis Found : C,65.30; H, 6.16; N, 4.13;

$C_{35}H_{36}N_2O_5$ $C_4H_4O_4$.2 H_2O Requires : C, 65.35; H, 6.18; N, 3.90%.

Example 97

N-[4-(4-((4-Fluorobenzyl)methylamino)butyl)phenyl]-9-oxo-4-thioxanthenecarboxamide

The coupling of 9-oxo-4-thioxanthenecarboxylic acid (0.72g) with 4-amino-N-[(4-fluorophenyl)methyl]-N-methylbenzenebutanamine (Intermediate 33(i) in EP-A-494623) (0.86g) gave, after crystallisation from ethanol, the title compound as a solid (0.37g), mp = 168°.

Analysis Found : C,72.54; H,5.57; F,3.62; N,5.92; S,5.76;

$C_{32}H_{29}F_1N_2O_2S_1$ Requires : C,73.26; H,5.57; F,3.62; N,5.34; S,6.11%.

Example 98

N-[2-Methyl-4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)propoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (1g) with Intermediate 11(c) (1.46g) gave the title compound as an oil (0.86g), fumarate (from isopropanol), mp = 94°.

Analysis Found : C,65.30; H,6.16; N,4.13;

$C_{35}H_{36}N_2O_5$, $C_4H_4O_4$, $2H_2O$ Requires : C,65.34; H,6.18; N,3.90%.

Example 99

Fumarate of N-[4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-2-hydroxypropoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.5g) with Intermediate 13 (0.79g) gave the titl compound (0.7g) as a solid, mp = 160°.

Analysis Found : C,66.92; H,5.57; N,4.05;

$C_{34}H_{34}N_2O_6\ C_4H_4O_4$ Requires : C,66.85; H,5.61; N,4.10%.

Example 100

Fumarate of N-[4-[3-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-2-hydroxypropoxy]phenyl]-3-(4-fluorobenzoyl)benzamide

The coupling of Intermediate 15 (0.36g) with Intermediate 13 (0.44g) gave the title compound (0.2g) as a solid, mp = 162 - 164°.

Analysis Found : C,65.15; H,5.41; F,2.65; N,4.05;

$C_{34}H_{33}FN_2O_6\ C_4H_4O_4$ Requires : C,65.14; H,5.32; F,2.71; N,4.00%.

Example 101

Oxalate of N-[4-[3-(methylbenzylamino)propoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.7g) with Intermediate 11(e) (0.83g) gave the title compound (1.1g) as a solid, mp = 172°.

Analysis Found : C,69.92; H,5.69; N,4.94;

$C_{31}H_{30}N_2O_3\ C_2H_2O_4$ Requires : C,69.71; H,5.67; N,4.93%.

Example 102

Oxalate of N-[4-[3-(1,2,3,4-tetrahydro-2-isoquinolinyl)propoxy]phenyl]-3-benzoylbenzamide

The coupling of 3-benzoylbenzoic acid (0.4g) with 4-[3-(1,2,3,4-tetrahydro-2-isoquinolinyl)propoxy]benzenamine (Intermediate 88 in EP-A-494623) (0.5g) gave the title compound (0.37g) as a solid, mp = 180°.

Analysis Found : C,70.21; H,5.57; N,4.88;

$C_{32}H_{30}N_2O_3\ C_2H_2O_4$ Requires : C,70.33; H,5.56; N,4.82%.

Example 103

N-[4-(2-(Benzylmethylamino)ethoxy)phenyl]-3-benzoylbenamide

The coupling of 3-benzoylbenzoic acid (0.8g) with Intermediate 19 (0.9g) gave the title compound as an oil (1.1g), hydrochloride (from diethyl ether), mp = 140°.

Analysis Found : C,71.35; H,5.85; Cl,6.91; N,5.43;

$C_{30}H_{27}N_2O_3$, HCl Requires : C,71.92; H,5.83; Cl, 7.08;

Example 104

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-4-oxo-2-phenyl-4H-1-benzopyran-8-carboxamide

A mixture of 4-oxo-2-phenyl-4H-1-benzopyran-8-carboxylic acid* (1g) and 1-hydroxybenzotriazole (0.58g) in DMF (50ml) was stirred at room temperature for 10 min. 4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (1.1g) was then added, followed by dicyclohexylcarbodiimide (0.67g) and the mixture was stirred at room temperature for 16 h and then filtered. The filtrate was concentrated in vacuo, treated with dilute sodium hydroxide solution and extracted with methylene chloride. The combined, dried, organic extracts were evaporated and the residue was purified by column chromatography on silica gel eluting with methylene chloride/methanol (99:1) to give the title compound (0.6g) as a white solid, after crystallisation from ethyl acetate, mp = 117-120°.

Analysis Found: C,74.40; H,6.22; N,4.63; O,14.49;

$C_{37}H_{36}N_2O_5$ $0.5H_2O$ Requires: C,74.35; H,6.24; N,4.68; O,14.72%

*Paolo Da Re E. Sianesi and V. Mancini, Chem. Ber., 1966, 99, 1962.

The following compounds were prepared in a similar manner:

Example 105

N-[4-(3-(Methylveratrylamino)propylthio)phenyl]-1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxamide

The coupling of 1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxylic acid* (0.68g) with N-[3-[(4-aminophenyl)thio]propyl]-3,4-dimethoxy-N-methylbenzenemethanamine (Intermediate 38(d) in EP-A-494623) (0.88g) gave, after crystallisation from isopropanol, the title compound as a solid (0.1g), mp = 130°.

Analysis Found : C, 70.89; H, 6.08; N, 6.98; S, 5.50;

$C_{35}H_{35}N_3O_4S_1$ Requires : C, 70.80; H, 5.94; N, 7.08; S, 5.40%.

*Graham J Atwell et al., J.Med.Chem. 1989, 32, 396-401.

Example 106

N-[4-(3-(Methylveratrylamino)propyl)phenyl]-1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxamide

The coupling of 1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxylic acid (0.89g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenepropanamine (Intermediate 33(f) in EP-A-494623) (0.9g) gave, after crystallisation from isopropanol, the title compound as a solid (0.47g), mp = 180°.

Analysis Found : C, 74.73; H, 6.28; N, 7.39;

$C_{35}H_{35}N_3O_4$ Requires : C, 74.84; H, 6.28; N, 7.48%.

Example 107

N-[4-(2-(Methylveratrylamino)ethoxy)phenyl]-1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxamide

The coupling of 1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxylic acid (0.8g) with N-[2-(4-aminophenoxy)ethyl]-3,4-dimethoxy-N-methylbenzenemethanamine

(Intermediate 36(b) in EP-A-494623) (0.95g) gave, after crystallisation from ethanol, the title compound as a solid (0.6g), mp = 175°.

Analysis	Found :	C, 72.50; H, 5.82; N, 7.45;
$C_{34}H_{33}N_3O_5$	Requires :	C, 72.45; H, 5.90; N, 7.45%.

Example 108

N-[4-(4-(Methylveratrylamino)butyl)phenyl]-1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxamide

The coupling of 1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxylic acid (0.8g) with 4-amino-N-[(3,4-dimethoxyphenyl)methyl]-N-methylbenzenebutanamine (Intermediate 33(a) in EP-A-494623) (0.52g) gave, after crystallisation from diisopropyl ether, the title compound as a solid (0.13g), mp = 171°.

Analysis	Found :	C, 72.11; H, 6.59; N, 6.89;
$C_{36}H_{37}N_3O_4$, H_2O	Requires :	C, 72.76; H, 6.57; N, 7.06%.

Example 109

N-[4-[2-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-oxo-2-phenyl-4H-1-benzopyran-8-carboxamide

The coupling of 4-oxo-2-phenyl-4H-1-benzopyran-8-carboxylic acid (0.5 g) with 4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]benzenamine (Intermediate 2(c) in EP-A-494623) (0.58 g) gave, after crystallisation from acetonitrile, the title compound (0.3 g) as a solid, mp 135-140°.

Analysis	Found :	C,73.17; H,5.78; N,4.87; O,16.38;
$C_{35}H_{32}N_2O_5$ 0.75H_2O	Requires :	C,73.21; H,5.88; N,4.85; O,16.02%.

Example 110

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-2-(3-methoxyphenyl)-4-oxo-4H-1-benzopyran-8-carboxamide

The coupling of 2-(3-methoxyphenyl)4-oxo-4H-1-benzopyran-8-carboxylic acid (0.5g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.52 g) gave, after crystallisation from ethyl acetate, the title compound (0.45 g) as a solid, mp = 152°.

Analysis Found : C,73.22; H,6.21; N,4.44; O,16.09;

$C_{38}H_{38}N_2O_6$ $0.25H_2O$ Requires : C,73.23; H,6.22; N,4.49; O,16.04%.

Example 111

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxamide

The coupling of 1,4-dihydro-4-oxo-2-phenyl-8-quinolinecarboxylic acid (0.4 g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.47 g) gave, after crystallisation from isopropanol, the title compound (100 mg) as a solid, mp = 204°.

Analysis Found : C,75.01; H,6.31; N,7.01; O,11.60;

$C_{37}H_{37}N_3O_4$ $0.25H_2O$ Requires : C,75.04; H,6.38; N,7.09; O,11.48%.

Example 112

N-[4-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]phenyl]-1,4-dihydro--2-(3-methoxyphenyl)-4-oxo-8-quinolinecarboxamide

The coupling of 1,4-dihydro-2-(3-methoxyphenyl)-4-oxo-8-quinolinecarboxylic acid (0.22g) with 4-[4-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)butyl]benzenamine (Intermediate 2(d) in EP-A-494623) (0.25 g) gave, after crystallisation from ethyl acetate, the titl compound (50 mg) as a solid, mp = 116°.

Analysis Found : C,71.32; H,6.45; N,6.63;

$C_{38}H_{39}N_3O_5$ 1.25H_2O Requires : C,71.28; H,6.53; N,6.56%.

Example 113

In vitro cytotoxicity of MDR inhibitors in Chinese Hamster Ovary cells

The multidrug resistant Chinese Hamster Ovary (CHO) cell line CH^RC5 was obtained from Dr V Ling, Princess Margaret Hospital, Toronto, Canada and maintained as anchorage-dependent monolayers in α-minimum essential medium supplemented with thymidine, adenosine, 10% fetal bovine serum, 2mM L-glutamine (Flow), 100 units/ml penicillin and 100μg/ml streptomycin in a humidified atmosphere of 95% air and 5% carbon dioxide. Cells were passaged into culture flasks twice a week after dissociation with EDTA.

CH^RC5 cells were seeded at a density of 10^4 cells/well in microtitre plates. After 24 hours, the medium was removed and replaced by 0.1mL of fresh medium containing successive two-fold dilutions of MDR inhibitors. Each MDR inhibitor was assayed in duplicate in two-fold dilution from 1250 to 20nM. The last well of each column was utilised to verify the lack of toxicity at the top dose of the MDR inhibitor in the absence of doxorubicin. Other control conditions were assayed on each microtitre plate : cells alone (1 well), doxorubicin alone (7 wells), amiodarone (a range of two-fold dilutions starting at 5μM; two wells each). 0.1ml of a 10μg/ml solution of doxorubicin was added. After 72 hours incubation cell viability was assessed by the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) to a dark blue formazan product. In particular, 20μl of a 5mg/ml solution of MTT prepared in phosphate buffered saline was added to each well. After 4 hours incubation at 37°, the medium was aspirated and replaced with 0.1ml dimethylsulphoxide. After vigorous shaking, the quantity of formazan product formed was assessed by its optical density at 550nm. The absorbance is directly related to the number of surviving cells in the wells.

Cytotoxicity calculations were performed on the average of the two wells for each condition. The concentration of ach MDR inhibitor giving a 50%

reduction of the optical density relative to cells treated with doxorubicin alone was determined to give an EC_{50} value.

Results

In the above test the compounds of the specific Examples hereinabove had EC_{50} values of less than 1μM and are therefore more potent than prototype MDR inhibitors including amiodarone (EC_{50} 3μM) and verapamil (3μM).

The following are examples of pharmaceutical compositions according to the invention. The term 'Active Ingredient' as used hereinafter means a compound of the invention and may be for example a compound of Examples 1-112.

Example A - Oral Tablet

	Per Tablet (mg)
Active Ingredient	50.0
Microcrystalline Cellulose	110.0
Lactose	67.5
Sodium Starch Glycolate	20.0
Magnesium Stearate	2.5
Total	250.0

The drug is sieved through a 250μm sieve and then the five powders are intimately mixed in a blender and compressed on 3/8 inch standard concave punches in a tabletting machine.

Example B - Oral Capsule

	Per Capsule (mg)
Activ Ingredient	50.0
Microcrystalline C llulose	66.5

Lactose USP	66.5
Sodium Starch Glycolate	15.0
Magnesium Stearate	2.0
Total	200.0

The drug is sieved through a 250μm sieve and then the five powders are intimately mixed in a blender and filled into No. 2 hard gelatin capsule shells on a capsule filling machine.

Example C - Injection for Intravenous Administration (10mg in 10mL)

	% w/w
Active Ingredient	0.1
Cancer chemotherapy agent	as required
Water for Injection to	100.0
Dilute hydrochloric acid to	pH 3.0

The active ingredient (and cancer chemotherapy agent where appropriate) is dissolved with mixing in the Water For Injection, adding acid slowly until the pH is 3.0. The solution is sparged with nitrogen and filtratively sterilized through a sterilized filter of 0.22 micron pore size. Under aseptic conditions this sterile solution is placed into sterile ampoules and the ampoules flame sealed.

Example D - Oral Syrup

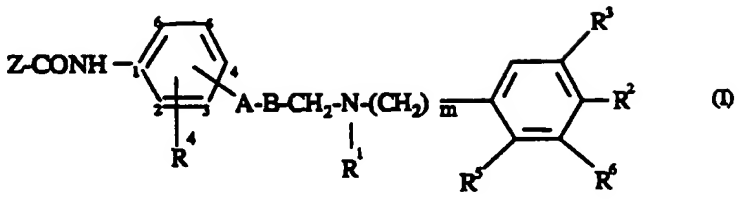
	% w/v
Active Ingredient	2.0
Cancer chemotherapy agent	as required
Dilute hydrochloric acid to	pH 3.0
Sobitol solution	60 v/v

Flavour	as required
Distilled water to	100

The active ingredient (and cancer chemotherapy agent where appropriate) is dissolved in some of the water with stirring by adding gradually the hydrochloric acid until the pH is 3.0. The sorbitol solution, flavour and the rest of the water are added and the pH re-adjusted to 3.0. The syrup is clarified by filtration through suitable filter pads.

CLAIMS

1. A compound of formula (I):



(I)

and salts and solvates thereof, including physiologically acceptable salts and solvates thereof, in which:

A represents an oxygen or a sulphur atom, a bond or a group $(CH_2)_lNR^7$ (where l represents zero or 1 and R^7 represents a hydrogen atom or a methyl group);

B represents a C_{1-4} alkylene chain optionally substituted by a hydroxyl group, except that the hydroxyl group and moiety A cannot be attached to the same carbon atom when A represents an oxygen or sulphur atom or a group $(CH_2)_lNR^7$, or when A represents a bond B may also represent a C_{2-4} alkenylene chain;

R^1 represents a hydrogen atom or a C_{1-4} alkyl group;

m represents 1 or 2;

R^2 represents a hydrogen or a halogen atom, or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group;

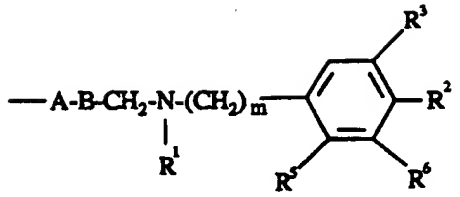
R^3 represents a hydrogen atom or a C_{1-4} alkoxy group;

R^4 represents a hydrogen atom or a C_{1-4} alkyl or C_{1-4} alkoxy group;

R^5 represents a hydrogen atom or R^1 and R^5 together form a group $-(CH_2)_n-$ where n represents 1 or 2;

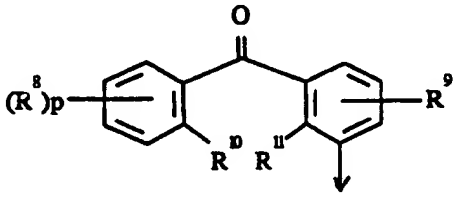
R^6 represents a hydrogen atom or a C_{1-4} alkoxy group;

the group

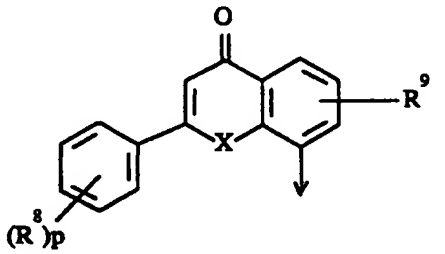


is attached at the benzene ring 3 or 4 position relative to the carboxamide substituent, provided that when the group is attached at the benzene ring 3 position then R^4 must be attached at the benzene ring 6 position; and

Z represents either Het,



or



Het represents an optionally substituted bicyclic or tricyclic ring selected from quinolin-4-yl, isoquinolin-1-yl, isoquinolin-3-yl, quinolin-3-yl, quinolin-2-yl, quinoxalin-2-yl, naphthalen-1-yl, naphthalen-2-yl, indol-2-yl, 4-oxo-4H-1-benzopyran-2-yl, phenazin-1-yl and phenothiazin-1-yl or an aryl substituted monocyclic ring selected from 2-aryl-4-thiazolyl, 2-aryl-5-thiazolyl, 5-aryl-2-thienyl, 2-aryl-4-triazolyl and 1-aryl-4-pyrazolyl where aryl represents a phenyl or pyridyl ring optionally substituted by a halogen atom or a trifluoromethyl, C_{1-4} alkyl or C_{1-4} alkoxy group. The above mentioned bicyclic or tricyclic rings may be unsubstituted or substituted by one, two or three groups sel cted from C_{1-4} alkyl and C_{1-4} alkoxy. Quinolin-4-yl rings may also be substituted in the ring 2 position by phenyl or phenyl

substituted by C_{1-4} alkoxy. Indol-2-yl rings may also be substituted in the ring 3 position by benzoyl;

R^8 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylthio, amino or nitro group;

p represents 1; or when R^8 represents C_{1-4} alkoxy p may also represent 2 or 3;

R^9 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group;

R^{10} and R^{11} may each represent a hydrogen atom or together form a bond or a linking atom selected from -O- or -S-; and

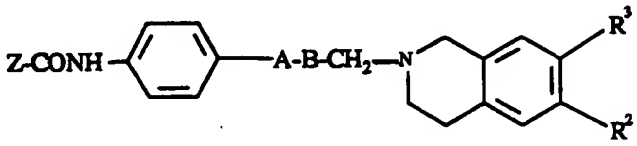
X represents an oxygen atom or NR^{12} (where R^{12} represents a hydrogen atom or a C_{1-4} alkyl group).

2. A compound according to Claim 1 in which R^2 and R^3 each represent a C_{1-4} alkoxy group and R^6 represents a hydrogen atom.

3. A compound according to Claim 1 or Claim 2 in which R^4 represents a hydrogen atom.

4. A compound according to any preceding claim in which m represents 1 and R^1 and R^5 together form a group $-(CH_2)_2-$.

5. A compound of formula (Ia).



(Ia)

wh rein Z is as d fined in Claim 1 above;

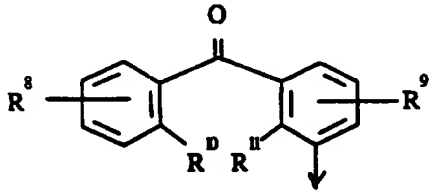
A repr sents an oxygen or a sulphur atom or a bond;

B represents an unsubstituted C_{1-4} alkylene chain;

R^2 and R^3 each independently represents a C_{1-4} alkoxy group; and physiologically acceptable salts and solvates thereof.

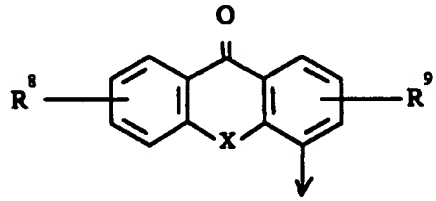
6. A compound according to Claim 5 in which Z represents Het as defined in Claim 1 above.

7. A compound according to Claim 5 in which Z represents



wherein R^8 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylthio or nitro group, R^9 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group and R^{10} and R^{11} are as previously defined in Claim 1.

8. A compound according to Claim 5 in which Z represents



wherein R^8 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylthio or nitro group, R^9 represents a hydrogen or halogen atom or a C_{1-4} alkyl, C_{1-4} alkoxy or C_{1-4} alkylthio group and X represents an oxygen atom or NH.

9. A compound according to Claim 7 or Claim 8 in which R^8 represents a hydrogen or fluorine atom or a C_{1-4} alkoxy or C_{1-4} alkyl group and R^9 represents a hydrogen atom.

10. A compound according to any preceding claim for use in therapy.

11. A compound according to any of Claims 1 to 9 for use in the treatment of a mammal which is suffering from cancer, to improve or increase the efficacy of an anti-tumour drug, or increase or restore sensitivity of a tumour to an anti-tumour drug, or reverse or reduce resistance of a tumour to an anti-tumour drug.

12. Use of a compound according to any of Claims 1 to 9 for the manufacture of a medicament for the treatment of a mammal suffering from cancer, to improve or increase the efficacy of an anti-tumour drug, or increase or restore sensitivity of a tumour to an anti-tumour drug, or reverse or reduce resistance of a tumour to an anti-tumour drug.

13. A method of treatment of a mammal which is suffering from cancer, which method comprises administering to said mammal an effective amount of a compound according to any of Claims 1 to 9 to improve or increase the efficacy of an anti-tumour drug, or increase or restore sensitivity of a tumour to an anti-tumour drug, or reverse or reduce resistance of a tumour to an anti-tumour drug.

14. A pharmaceutical composition which comprises a compound according to any of Claims 1 to 9 together with one or more physiologicaliy acceptable carriers or excipients.

15. A pharmaceutical composition which comprises an active amount of a compound according to any of Claims 1 to 9 for use in the treatment of a mammal which is suffering from cancer, to improve or increase the efficacy of an anti-tumour drug, or increase or restore sensitivity of a tumour to an anti-tumour drug, or reverse or reduce resistance of a tumour to an anti-tumour drug.

16. A pharmaceutical composition according to Claim 14 or 15 in a form suitable for oral, buccal, parenteral or rectal administration.

17. A pharmaceutical composition according to any of Claims 14 to 16 in unit dosage form.

18. A product containing a compound according to any of Claims 1 to 9 and an anti-tumour drug as a combined preparation for simultan ous, separat or sequential use in treating cancer.

19. A compound according to any of Claims 1 to 9 and an anti-tumour drug in the presence of each other in the human or non-human animal body for use in treating cancer.

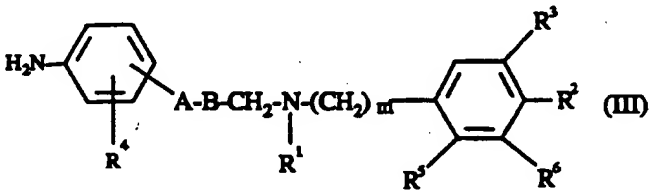
20. Product or process according to any of Claims 11 to 19 (except Claim 14) wherein the anti-tumour drug is selected from Vinca alkaloids, anthracyclines, taxol and derivatives thereof, podophyllotoxins, mitoxantrone, actinomycin, colchicine, gramicidine D, amsacrine or any drug having cross-resistance with the above drugs characterised by the so-called MDR phenotype.

21. A process for the preparation of a compound according to Claim 1 which comprises :

(A) reacting a compound of formula (II)

(II)

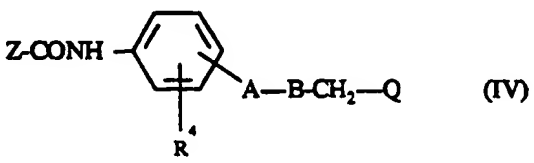
with a compound of formula (III)



(III)

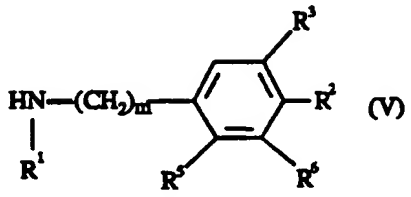
in the presence of a coupling reagent; or

(B) reacting a compound of formula (IV)



(IV)

(wherein Q represents a halogen atom) with a compound of formula (V)



or a salt thereof in the presence of an acid acceptor; with salt formation as an optional step subsequent to process (A) or (B).

22. Compounds according to any of Claims 1 to 9 substantially as herein described.

23. Compositions according to any of Claims 14 to 17 substantially as herein described.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/01802

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D217/04; A61K31/47; C07D401/12; C07D335/16
C07C235/84; C07C233/80; C07D215/48; C07D215/52 ./.

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; C07C

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0 206 802 (DEVELOPMENT FINANCE CORPORATION OF NEW ZEALAND) 30 December 1986 see claims 1,12-15 ---	1,10-20
A	EP,A,0 172 744 (DEVELOPMENT FINANCE CORPORATION OF NEW ZEALAND) 26 February 1986 see claims 1,20-23 ---	1,12-20
A	JOURNAL OF MEDICINAL CHEMISTRY vol. 31, no. 3, March 1988, WASHINGTON US pages 707 - 712 BRIAN D. PALMER ET AL 'Potential antitumor agents.54.Chromophore requirements for in vivo antitumor activity among the general class of linear tricyclic carboxamides' --- -/--	1,12-20

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 SEPTEMBER 1993	-4. 10. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HENRY J.C.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	EP,A,0 494 623 (LABORATOIRES GLAXO SA) 15 July 1992 cited in the application see claims	1,12-20

INTERNATIONAL SEARCH REPORT

International Application N PCT/EP 93/01802

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IPC[5]: C07D311/86; C07D217/26; C07D241/46

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]

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International Searching Authority	**Signature of Authorized Officer**
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 93/01802

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 13 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compounds.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9301802
SA 76742

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent ffice EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0206802	30-12-86	JP-A- 62048669	03-03-87
		US-A- 4904659	27-02-90
EP-A-0172744	26-02-86	JP-A- 61112061	30-05-86
EP-A-0494623	15-07-92	AU-A- 1154392	17-08-92
		WO-A- 9212132	23-07-92

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(51) International Patent Classification 6 : A61K 45/06, 38/13

A1

(11) International Publication Number: **WO 95/20980**

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(22) International Filing Date: 11 January 1995 (11.01.95)

(30) Priority Data:
08/190,288 2 February 1994 (02.02.94) US

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(74) Agent: BERLINER, Robert; Robbins, Berliner & Carson, 5th floor, 201 N. Figueroa Street, Los Angeles, CA 90012 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR INCREASING BIOAVAILABILITY OF ORAL PHARMACEUTICAL COMPOSITIONS

(57) Abstract

A method for increasing bioavailability of an orally administered hydrophobic pharmaceutical compound, which comprises orally administering the pharmaceutical compound to a mammal in need of treatment with the compound concurrently with a bioenhancer comprising an inhibitor of a cytochrome P450 3A enzyme or an inhibitor of P-glycoprotein-mediated membrane transport, the bioenhancer being present in sufficient amount to provide bioavailability of the compound in the presence of the bioenhancer greater than the bioavailability of the compound in the absence of the bioenhancer.

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METHOD FOR INCREASING BIOAVAILABILITY OF ORAL PHARMACEUTICAL COMPOSITIONS

This invention was made with Government support under Grant No. GM 26691, awarded by the National Institutes of Health. The Government has certain rights in this invention.

INTRODUCTION

Technical Field

This invention is directed to the field of pharmacology and particularly to the formulation of pharmaceutical compositions for increased bioavailability.

Background

Bioavailability

Pharmacokinetics is the study of the fate of pharmaceuticals from the time they are ingested until they are eliminated from the body. The sequence of events for an oral composition includes absorption through the various mucosal surfaces, distribution via the blood stream to various tissues, biotransformation in the liver and other tissues, action at the target site, and elimination of drug or metabolites in urine or bile.

Bioavailability of a drug (pharmaceutical composition) following oral dosing is a critical pharmacokinetic determinant which can be approximated by the following formula:

$$F_{oral} = F_{ABS} \times F_G \times F_H$$

F_{oral} is oral bioavailability fraction, which is the fraction of the oral dose that reaches the circulation in an active, unchanged form. F_{oral} is less than 100% of the active ingredient in the oral dose for three reasons: drug is not absorbed through the GI tract and is eliminated in the feces; drug is biotransformed by the cells of the intestine (to an inactive metabolite); or drug is eliminated by the cells of the liver, either by biotransformation and/or by transport into the bile. Thus, oral bioavailability is the product of the fraction of the oral dose that is absorbed

(F_{ABS}), the fraction of the absorbed dose that successfully reaches the blood side of the gastrointestinal tract (F_G), and the fraction of the drug in the GI blood supply that reaches the heart side of the liver (F_H). Previous drug formulations have attempted to increase drug efficacy by increasing drug absorption. For example, methods have been used to increase drug absorption using liposomes as carriers and designing more lipophilic drugs. These methods can increase drug absorption; however, they fail to address other ways of increasing drug bioavailability.

Liver Biotransformation and Biliary Secretion

The liver affects drug bioavailability. All blood from the gastrointestinal tract passes through the liver before going elsewhere in the body in all mammals, including humans. Due to its location, liver transformation of orally dosed drugs has a substantial "first-pass effect" on drug bioavailability that was thought to exceed effects in the gut, as discussed by Yun K. Tam in "Individual Variation in First-Pass Metabolism," Clin. Pharmacokinetics 25:300-328 (1993):

> Enzyme activity in the small intestine is lower than in the liver. In humans, the liver to intestine cytochrome P450 ratio has been reported as ≈20, suggesting that the contribution of intestinal phase I biotransformation to the overall metabolism of a drug is unlikely to be important. (op. cit. 303)

Elimination of active drug by the liver occurs by one or both of two general pathways, namely biotransformation of the drug and excretion of the drug into the bile. Biotransformation reactions have been classified into two broadly defined phases. Phase I biotransformation often utilizes reactions catalyzed by the cytochrome P450 enzymes, which are manifold and active in the liver and transform many chemically diverse drugs. A second biotransformation phase can add a hydrophilic group, such as glutathione, glucuronic acid or sulfate, to increase water solubility and speed elimination through the kidneys.

Hepatocytes have contact with many types of blood and other fluid-transport vessels, such as the portal vein (nutrient and drug-rich blood from the gut), the hepatic arteries (oxygenated blood direct from the heart), the hepatic veins (efflux),

lymphatics (lipids and lymphocytes), and bile ducts. The biliary ducts converge into the gall bladder and common bile duct that excretes bile into the upper intestine, aiding digestion. Bile also contains a variety of excretory products including hydrophobic drugs and drug metabolites.

Traditional solubility rate limiting approaches to increasing drug efficacy have focused on increasing solubility and membrane permeability. Where metabolism-based approaches have been considered, they have focused on biotransformation in liver. Although methods exist that affect biotransformation in the liver, these methods are inadequate because they affect general liver metabolism and can produce broad non-specific systemic effects.

Cytochromes

Most biotransformation is performed by enzymes called "mixed function oxidases" containing cytochromes, molecules with iron-containing rings, that help reduce oxygen to water. The cytochrome-containing enzymes that transform drugs use radical oxygen. When oxygen is reduced to its reactive radical form, it reacts immediately with the drug at the oxygen reduction site.

Most research on cytochromes involved in drug biotransformation focuses on inter-individual differences in cytochrome activity because such differences appear to be the dominant mechanism for differences in elimination of pharmaceuticals between individuals. Large inter-individual differences observed in the effects of drugs are at least in part determined by the variation of the expression and catalytic activity of the cytochromes P450.

The sources of the inter-individual variation in the catalytic activity of the cytochromes P450 can be divided into four general categories. The first is the influence of genetics on the expression of the cytochromes P450. Significant inter-individual variability can occur for each of the cytochromes P450. Genetic polymorphisms have been well characterized for the two cytochromes P450 responsible for debrisoquine/sparteine metabolism (*CYP*2D6; cytochrome families are defined below) and (S)-mephenytoin 4'-hydroxylation (possibly *CYP*2C19). The second source of inter-individual differences is that several of the human cytochromes P450 are inducible. That is, the content as well as the catalytic

activity of these cytochromes P450 is increased by exposure of an individual to particular classes of drugs, endogenous compounds, and environmental agents. Thirdly, the activity of the cytochromes P450 can be inhibited or the cytochromes P450 can be inactivated by drugs and environmental compounds. This includes competitive inhibition between substrates of the same cytochrome P450, inhibition by agents that bind sites on the cytochrome P450 other than the active site, and suicidal inactivation of the cytochrome P450 by reactive intermediates formed during the metabolism of an agent. Another source of inter-individual differences is host factors. These factors include disease states, diet, and hormonal influences. Inter-individual differences in the level of expression and catalytic activity of the various cytochromes P450 can result in an altered response to a drug (individuals can be hypo- or hyper-responsive), a toxic response to unusual levels of a drug or metabolite, and individual sensitivity to chemical carcinogens.

Multiple Drug Resistance

Cancer cells that become resistant to one chemotherapeutic drug often become resistant to an entire group of chemotherapeutic drugs. This phenomenon is usually called multiple drug resistance (MDR).

Many patients on chemotherapy initially have striking remissions but later relapse and die from cancer that exhibits resistance to a wide array of structurally unrelated antineoplastic agents. The MDR phenomenon includes cross-resistance among the anthracyclines, the epipodophyllotoxins, the vinca alkaloids, taxol, and other compounds. A number of drugs are able to reverse MDR, including calcium channel blockers, phenothiazines, quinidine, antimalarial agents, antiestrogenic and other steroids, and cyclosporine. Liposome therapy also reverses MDR, with or without a drug on board.

In vitro studies in the past indicate that this form of resistance is associated with amplification or over-expression of the *mdr-1* gene in tumors. The *mdr-1* gene codes for the expression of a cell surface protein, P-glycoprotein (P-gp), a transmembrane protein which acts as an energy-dependent efflux pump that transports drugs associated with MDR out of the tumor cell before cytotoxic effects

occur. ATP hydrolysis on the cytoplasmic face of P-gp is required for export of hydrophobic compounds from a tumor cell.

Normal *mdr-1* expression occurs in secretory epithelial cells of the liver, pancreas, small intestine, colon, and kidney; endothelial capillary cells of the brain and testis; placental trophoblasts; and the adrenal gland. In the liver, P-gp is localized on the biliary domain of the hepatocyte membrane. In the small intestine and colon, P-gp is present on the luminal side of epithelial cells. P-gp transports dietary toxins back into the lumen and therefore helps prevent the toxins from being absorbed into the portal circulation.

Clinical studies have also previously shown that pharmaceuticals that are effective in eliminating MDR of tumor cells *in vitro* (apparently by inhibition of P-gp) restore chemotherapeutic cytotoxicity *in vivo*. Studies with small numbers of patients suggest that the addition of verapamil, diltiazem, quinine, trifluoperazine, or cyclosporine to chemotherapeutic regimens has the potential to reverse MDR.

Absorption By The Gut

Absorption across epithelia, in particular intestinal epithelia, also affects drug bioavailability. The intestine lumen presents a convoluted surface that increases the surface area of the intestine to facilitate absorption of both nutrients and drugs. The membrane of the enterocyte contains many transport proteins that actively carry nutrients from the lumen of the gut into the interior of the enterocytes. Many molecules, including many drugs, passively diffuse or are actively transported through the membrane and into the cytoplasm. Most nutrients and drugs pass through the enterocyte and eventually diffuse into the capillary net on route to the portal circulation system and the liver.

The intestine can also pump drugs out of the intestine and back into the lumen. The ability of the intestine to pump drugs out of the tissue has been thought to be important in protection against potentially damaging hydrophobic cations and toxins and for protection against small intestine cancer. No drugs or formulations have been designed to reduce pumping of drugs back into the intestine to increase drug bioavailability prior to the present invention.

Relevant Literature

Back, D.J. and M.L. Orme. "Pharmacokinetic drug interactions with oral contraceptives." Clin Pharmacokinet 18 (6 1990):472-84.

Back, D.J. and S.M. Rogers. "Review: first-pass metabolism by the gastrointestinal mucosa." Aliment Pharmacol Ther 1 (5 1987): 339-57.

Benet, L.Z. and R.L. Williams. "Appendix II: Design and optimization of dosage regimes; pharmacokinetic data." In Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds. Alfred Gilman, Theodore W. Rall, Alan S. Nies, and Palmer Taylor, Eighth ed., New York: Pergamon Press, 1990.

Callaghan, R. and J. R. Riordan. "Synthetic and natural opiates interact with P-glycoprotein in multidrug-resistant cells." J. Biol Chem 268 (21 1993): 16059-64.

Clynes, M. "Cellular models for multiple drug resistance in cancer." In Vitro Cell Dev Biol 3 (1 1993): 171-9.

Endicott, J.A. and V. Ling. "The biochemistry of P-glycoprotein-mediated multidrug resistance." Annu Rev Biochem 58 (71 1989): 137-71.

Fahr, A. "Cyclosporin clinical pharmacokinetics." Clin Pharmacokinetic 24 (6 1993): 472-95.

Fairchild, C.R. and K.H. Cowan. "Keynote address: multidrug resistance: a pleiotropic response to cytoxic drugs." Int J Radiat Oncol Biol Phys 20 (2 1991): 361-7.

Fasco, M.J., J.B. Silkworth, D.A. Dunbar, and L.S. Kaminsky. "Rat small intestinal cytochromes P450 probed by warfarin metabolism." Mol Pharmacol 43 (2 1993): 226-33.

Fojo, A.T. "Multidrug resistance." Adv Intern Med 36 (218 1991): 195-218.

Gatmaitan, Z.C. and I.M. Arias. "Structure and function of P-glycoprotein in normal liver and small intestine." Adv Pharmacol 24 (97 1993): 77-97.

Gilman, A.G., T.W. Rall, A.S. Nies, and P. Taylor. Goodman and Gilman's The Pharmacological Basis of Therapeutics. Eighth ed., New York: Pergamon Press, 1990.

Gomez, D., M. Hebert, and L.Z. Benet. "The effect of ketoconazole on the intestinal metabolism and bioavailability of cyclosporine" Clin Pharmacol Ther 55 (2 1994): in press.

Greenblatt, D.J. "Presystemic extraction: mechanisms and consequences." J Clin Pharmacol 33 (7 1993): 650-6.

Hait, W.N., J.F. Gesmonde, J.R. Murren, J.M. Yang, H.X. Chen, and M. Reiss, "Terfenadine (Seldane): a new drug for restoring sensitivity to multidrug resistant cancer cells." Biochem Pharmacol 45 (2 1993): 401-6.

Hebert, M.F., J.P. Roberts, T. Prueksaritanont, and L.Z. Benet. "Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction." Clin Pharmacol Ther 52 (5 1992): 453-7.

Hsing, S., Z. Gatmaitan, and J.M. Arias. "The function of Gp170, the multidrug-resistance gene product, in the brush border of rat intestinal mucosa." Gastroenterology 102 (3 1992): 879-85.

Hunter, J., B.H. Hirst, and N.L. Simmons. "Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers." Pharm Res 10 (5 1993): 743-9.

Jancis, E.M., R. Carbone, K.J. Loechner, and P.S. Dannies. "Estradiol induction of rhodamine 123 efflux and the multidrug resistance pump in rat pituitary tumor cells." Mol Pharmacol 43 (1 1993): 51-6.

Kaminsky, L.S. and M.J. Fasco. "Small intestinal cytochromes P450." Crit Rev Toxicol 21 (6 1991): 407-22.

Kolars, J.C., P. Schmiedlin-Ren, J.D. Schuetz, C. Fang, and P.B. Watkins. "Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes." J Clin Invest 90 (5 1992a): 1871-8.

Kolars, J.C., P. Schmiedlin-Ren, W.O. 3rd Dobbins, J. Schuetz, S.A. Wrighton, and P.B. Watkins. "Heterogeneity of cytochrome P450IIIA expression in rat gut epithelia." Gastroenterology 102 (4 Pt 1 1992b): 1186-98.

Kolars, J.C., P.L. Stetson, B.D. Rush, M.J. Ruwart, R.P. Schmiedlin-Ren, E.A. Duell, J.J. Voorhees, and P.B. Watkins. "Cyclosporine metabolism by P450IIIA in rat enterocytes--another determinant of oral bioavailability?" Transplantation 53 (3 1992c): 596-602.

Komori, M., T. Hashizume, H. Ohi, T. Miura, M. Kitada, K. Nagashima, and T. Kamataki. "Cytochrome P-450 in human liver microsomes: high-performance liquid chromatographic isolation of three forms and their characterization." J. Biochem 104 (6 1988): 912-6.

Kralovanszky, J., F. Harrington, A. Greenwell, and R. Melnick. "Isolation of viable intestinal epithelial cells and their use for in vitro toxicity studies." In Vivo 4 (3 1990): 201-4.

Kronbach, T.V., Fischer, and U.A. Meyer. "Cyclosporine metabolism in human liver: identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs." Clin Pharmacol Ther 43 (6 1988): 630-5.

Kronbach, T.D. Mathys, M. Umeno, F.J. Gonzalez, and U.A. Meyer. "Oxidation of midazolam and triazolam by human liver cytochrome P450IIIA4." Mol Pharmacol 36 (1 1989): 89-96.

Lalka, D., R. K. Griffith, and C.L. Cronenberger. "The hepatic first-pass metabolism of problematic drugs." J Clin Pharmacol 33 (7 1993): 657-69.

Ludescher, C., Gattringer, J. Drach, J. Hofmann, and H. Grunicke. "Rapid functional assay for the detection of multidrug-resistant cells using the fluorescent dye rhodamine 123 [letter; comment]." Blood 78 (5 1991): 1385-7.

Ludescher, C., J. Thaler, D. Drach, J. Drach, M. Spitaler, C. Gattringer, H. Huber, and J. Hofmann. "Detection of activity of P-glycoprotein in human tumor samples using rhodamine 123." Br J Haematol 82 (1 1992): 161-8.

Lum, B.L., G.A. Fisher, N.A. Brophy, A.M. Yahanda, K.M. Adler, S. Kaubisch, J. Halsey, and B.I. Sikie. "Clinical trials of modulation of multidrug resistance. Pharmacokinetic and pharmacodynamic considerations." Cancer 72 (11 Suppl 1993a): 3502-14.

Lum, B.L., M.P. Gosland, S. Kaubisch, and B.I. Sikic. "Molecular targets in oncology: implications of the multidrug resistance gene." Pharmacotherapy 13 (2 1993): 88-109.

Muranishi, S. "Absorption enhancers." Crit Rev Ther Drug Carrier Syst 7 (1 1990): 1-33.

Nielsen, D. and T. Skovsgaard. "P-glycoprotein as multidrug transporter: a critical review of current multidrug resistant cell lines." Biochim Biophys Acta 1139 (3 1992): 169-83.

Pearce, H.I., A.R. Safa, N.J. Bach, M.A. Winter,M.C. Cirtain, and W.T. Beck. "Essential features of the P-glycoprotein pharmacophore as defined by a series of reserpine analogs that modulate multidrug resistance." Proc Natl Acad Sci USA 86 (13 1989): 5128-32.

Pichard, L., I. Fabre, G. Fabre, J. Domergue, B. Saint Aubert, G. Mourad, and P. Maurel. "Cyclosporine A drug interactions. Screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes." Drug Metab Dipos 18 (5 1990): 595-606.

Schmiedlin-Ren, P., P.E. Benedict, W.O. 3rd Dobbins, M. Ghosh, J.C. Kolars, and P.B. Watkins. "Cultured adult rat jejunal explants as a model for studying regulation of CYP3A." Biochem Pharmacol 46 (5 1993): 905-18.

Somberg, J., G. Shroff, S. Khosla, and S. Ehrenpreis. "The clinical implications of first-pass metabolism: treatment strategies for the 1990s." J Clin Pharmacol 33 (7 1993): 670-3.

Tam, Y.K. "Individual variation in first-pass metabolism." Clinical Pharmacokinetics 25 (4 1993): 300-328.

Thierry, A.R., D. Vige, S.S. Coughlin, J.A. Belli, A. Dritschilo, and A. Rahman. "Modulation of doxorubicin resistance in multidrug-resistant cells by liposomes." FASEB J (6 1993): 572-9.

van Hoogdalem, E.J., A.G. de Boer, and D.D. Breimer. "Intestinal drug absorption enhancement: an overview." Pharmacol Ther 44 (3 1989): 407-43.

Warren, L., J.-C. Jardiller, A. Malarska, and M.-G. Akeli: "Increased accumulation of drugs in multidrug-resistance cells induced by liposomes" *Cancer Research* 52:3241 (1992).

Watkins, P.B. "The role of cytochromes P-450 in cyclosporine metabolism." J Am Acad Dermatol 23 (6 Pt 2 1990): 1301-9.

Watkins, P.B. "Drug metabolism by cytochromes P450 in the liver and small bowel." Gastroenterology Clinics of North America 21 (3 1992): 511-526.

Watkins, Paul B. "Drug metabolism by cytochromes P450 in the liver and small bowel." Gastroenterology Clinics of North America 21 (3 1992): 511-526.

Watkins, P.B., S.A. Murray, L.G. Winkelman, D.M. Heuman, S.A. Wrighton, and P.S. Guzelian. "Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450. Studies in rats and patients." J Clin Invest 83 (2 1989): 688-97.

Watkins, P.B., S.A. Wrighton, E.G. Schuetz. D.T. Molowa, and P.S. Guzelian. "Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man." J Clin Invest 80 (4 1987): 1029-36.

West, I.C. "What determines the substrate specificity of the multi-drug-resistance pump?." Trends Biochem Sci 15 (2 1990): 42-6.

Wrighton, S.A., W.R. Brian, M.A. Sari, M. Iwasaki, F.P. Guengerich, J.L. Raucy, D.T. Molowa, and M. Vandenbranden. "Studies on the expression and metabolic capabilities of human liver cytochrome P450IIIA5 (HLp3)." Mol Pharmacol 38 (2 1990) 207-13.

Wrighton, S.A., M. Vandenbranden, J.C. Stevens, L.A. Shipley, B.J. Ring, A.E. Rettie, and J.R. Cashman. "In vitro methods for assessing human hepatic drug metabolism: their use in drug development." Drug Metabolism Reviews 25 (4 1993): 453-484.

Wu, C.-Y., M.F. Hebert, and L.Z. Benet. "Use of IV and oral drug levels from cyclosporine (CSA) with concomitant rifampin to differentiate gut absorption and metabolism." Pharm Res 10 Supp. (1993): S-345.

Zamora, J.M., H.L. Pearce, and W.T. Beck. "Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells." Mol Pharmacol 33 (4 1988): 454-62.

SUMMARY OF THE INVENTION

The invention is concerned with optimization of drug bioavailability. The invention maximizes drug bioavailability by increasing net drug absorption or decreasing drug biotransformation in the gut by using either cytochrome P450 drug metabolism inhibitors or P-glycoprotein (P-pg) drug transport inhibitors, both of which are called "bioenhancers" for the purposes of this invention.

An important object of the invention is inhibiting enzymes of the cytochrome P450 3A class (CYP3A) in the gut in preference to other locations, such as the liver, which was previously thought to be the primary site of drug metabolism. Another object of the invention is to inhibit P-gp-controlled back transport to increase the net transport of drugs through the enterocyte layer, causing an increase in the bioavailability of the drug, since the protein P-gp pumps drugs that have been transported into the cytoplasm of the enterocytes back into the lumen of the gut.

The invention is carried out by coadministration of one or more bioenhancers with a drug or drugs to increase drug bioavailability. The

compositions and methods of the present invention can be used to increase drug efficacy in humans and in other mammals. Although veterinary use is specifically contemplated, the primary use will be in human treatment. Administration schemes include, but are not limited to, use of oral and topical formulations in humans and use of similar formulations for livestock.

One specific object of the present invention is to reduce inter-individual variability of the systemic concentrations of the compound, as well as intra-individual variability of the systemic concentrations of the pharmaceutical compound being administered.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram of enterocyte cytochrome P450 3A drug metabolism and P-glycoprotein drug transport mechanisms that lead to decreased drug bioavailability.

Figure 2 is a graph showing the increase in cyclosporine (CYA) bioavailability caused by co-administration of ketoconazole (KC), a bioenhancer. Pre-IV indicates administration of CYA prior to IV administration of KC. Post-IV indicates administration of CYA after IV administration of KC. Pre-PO indicates oral administration of CYA prior to oral administration of KC. Post-PO indicates oral administration of CYA after oral administration of KC. The data illustrate an increase in integrated systemic drug concentrations over time due to the addition of a bioenhancer, as indicated by the increase in the area under the curve from pre-PO to post-PO which is markedly greater than the enhancement seen for the increase in the area under the curve (AUC) from pre-IV to post-IV.

DESCRIPTION OF SPECIFIC EMBODIMENTS

Bioenhancers Increase Drug Bioavailability

The present invention is based on a new discovery of various factors affecting drug bioavailability. "Drug bioavailability" is defined as the total amount of drug systemically available over time. The present invention increases drug

bioavailability by inhibiting drug biotransformation in the gut and/or by inhibiting active transport systems in the gut which decrease the net transport of drugs across gut epithelia. In either case the composition responsible for increased drug bioavailability is called a bioenhancer in this specification. It has been discovered that, in contrast to previous teachings about the primacy of liver metabolism, the gut is the primary location of drug transformation for many drugs, if not the majority of drugs dosed orally. Thus, bioenhancers specifically targeted to the gut provide a number of advantages, as described in detail below.

In general, the present invention provides a method for increasing the bioavailability of an orally administered pharmaceutical compound (particularly one which is hydrophobic), which comprises orally administering the pharmaceutical compound to a mammal in need of treatment concurrently with a bioenhancer comprising an inhibitor of a member of the cytochrome P450 3A enzyme family or an inhibitor of P-glycoprotein-mediated membrane transport (or both), the bioenhancer being present in sufficient amount to provide integrated systemic concentrations over time of the compound greater than the integrated systemic concentrations over time of the compound in the absence of the composition. Changes in the integrated systemic concentrations over time are indicated by the area under the curve (AUC) defined below. In preferred embodiments side effects are reduced by providing a bioenhancer that is active only (or primarily) in the gut, either because of its structure or because of deliberately selected concentration effects.

Bioavailability Measurements

The increase in drug bioavailability attributable to administration of the bioenhancer can be determined by measuring total systemic drug concentrations over time after coadministration of a drug and a bioenhancer and after administration of only the drug. The increase in drug bioavailability is defined as an increase in the Area Under the Curve (AUC). AUC is the integrated measure of systemic drug concentrations over time in units of mass-time/volume. The AUC from time zero (the time of dosing) to time infinity (when no drug remains in the

body) following the administration of a drug dose is a measure of the exposure of the patient to the drug. When efficacy of the bioenhancer is being measured, the amount and form of active drug administered should be the same in both the coadministration of drug and bioenhancer and the administration of the drug alone. For instance, administration of 10 mg of drug alone may result in total systemic drug delivered over time (as measured by AUC) of 500 μg-hr/ml. In coadministration (i.e., in the presence of the bioenhancer) the systemic drug AUC will increase to 700 μg-hr/ml. However, if significantly increased drug bioavailability in the presence of the bioenhancer is anticipated, drug doses may need to be reduced for safety. Systemic drug concentrations are measured using standard *in vitro* or *in vivo* drug measurement techniques. "Systemic drug concentration" refers to a drug concentration in a mammal's bodily fluids, such as serum, plasma or blood; the term also includes drug concentrations in tissues bathed by the systemic fluids, including the skin. Systemic drug concentration does not refer to digestive fluids. The increase in total systemic drug concentrations is one way of defining an increase of drug bioavailability due to coadministration of bioenhancer and drug. For drugs excreted unmetabolized in the urine, an increased amount of unchanged drug in the urine will reflect the increase in systemic concentrations.

Characteristics of Drugs Used With Bioenhancers

The word "drug" as used herein is defined as a chemical capable of administration to an organism which modifies or alters the organism's physiology. More preferably the word "drug" as used herein is defined as any substance intended for use in the treatment or prevention of disease. Drug includes synthetic and naturally occurring toxins and bioaffecting substances as well as recognized pharmaceuticals, such as those listed in "The Physicians Desk Reference," 47th edition, 1993, pages 101-321; "Goodman and Gilman's The Pharmacological Basis of Therapeutics" 8th Edition (1990), pages 84-1614 and 1655-1715; and "The United States Pharmacopeia, The National Formulary", USP XXII NF XVII (1990), the compounds of these references being herein incorporated by reference.

The term drug also includes compounds that have the indicated properties that are not yet discovered or available in the U.S. The term drug includes pro-active, activated and metabolized forms of drugs. The present invention can be used with drugs consisting of charged, uncharged, hydrophilic, zwitter-ionic, or hydrophobic species, as well as any combination of these physical characteristics. A hydrophobic drug is defined as a drug which in its non-ionized form is more soluble in lipid or fat than in water. Preferably, a hydrophobic drug is defined as a drug more soluble in octanol than in water.

Increased Drug Bioavailability by Inhibition of Cytochrome P450

Phase I Biotransformation

Reduction of enterocyte cytochromes P450 participation in drug biotransformation is one objective of the present invention. The major enzymes involved in drug metabolism are present in the endoplasmic reticulum of many types of cells but are at the highest concentration in hepatocytes. Traditionally, enterocyte biotransformation was considered of minor importance in biotransformation compared to the liver. Many compounds inhibit cytochrome P450. These include, but are not limited to, ketoconazole, troleandomycin, gestodene, flavones such as quercetin and naringenin, erythromycin, ethynyl estradiol, and prednisolone. The first goal of the invention is to use cytochrome P450 inhibitors to inhibit drug cytochrome P450 biotransformation in the gut to increase drug bioavailability.

Types Of Cytochromes And Tissue Location

The cytochromes P450 are a superfamily of hemoproteins. They represent the terminal oxidases of the mixed function oxidase system. The cytochrome P450 gene superfamily is composed of at least 207 genes that have been named based on the evolutionary relationships of the cytochromes P450. For this nomenclature system, the sequences of all of the cytochrome P450 genes are compared, and those cytochromes P450 that share at least 40% identity are defined as a family (designated by *CYP* followed by a Roman or Arabic numeral, *e.g. CYP*3), further

divided into subfamilies (designated by a capital letter, *e.g. CYP*3A), which are comprised of those forms that are at least 55% related by their deduced amino acid sequences. Finally, the gene for each individual form of cytochrome P450 is assigned an Arabic number (*e.g. CYP*3A4).

Three cytochrome P450 gene families (*CYP*1, *CYP*2 and *CYP*3) appear to be responsible for most drug metabolism. At least 15 cytochromes P450 have been characterized to varying degrees in the human liver. At concentrations of the substrates found under physiologic conditions, enzyme kinetics often favor a single form of cytochrome P450 as the primary catalyst of the metabolism of a particular drug or other enzyme substrate.

The *CYP*3 gene family encoding cytochromes P450 of type 3 is possibly the most important family in human drug metabolism. At least 5 forms of cytochrome P450 are found in the human 3A subfamily, and these forms are responsible for the metabolism of a large number of structurally diverse drugs. In non-induced individuals 3A may constitute 15% of the P450 enzymes in the liver. In enterocytes, members of the 3A subfamily constitute greater than 70% of the cytochrome-containing enzymes. The first two human 3A subfamily members identified were 3A3 and 3A4. These two cytochromes P450 are so closely related that the majority of studies performed to date have not been able to distinguish their contributions, and thus they are often referred to as 3A3/4. Erythromycin N-demethylation, cyclosporine oxidation, nifedipine oxidation, midazolam hydroxylation, testosterone 6β-hydroxylation, and cortisol 6β-hydroxylation are all *in vitro* probes of 3A3/4 catalytic activity. The levels of 3A3/4 vary by as much as 60-fold between human liver microsomal samples with the levels of 3A forms approaching 50% of the total cytochrome P450 present in human liver samples from individuals receiving inducers of 3A3/4. The recently studied CYP3A5 may also play a role as important as 3A3/4.

The liver contains many isoforms of cytochrome P450 and can biotransform a large variety of substances. The enterocytes lining the lumen of the intestine also have significant cytochrome P450 activity, and this activity is dominated by a single family of isozymes, 3A, the most important isoforms in drug metabolism.

Increased Drug Efficacy By Reducing CYP3A Drug Biotransformation

Preferred bioenhancers of the invention reduce drug biotransformation in the gut by inhibiting CYP3A activity in gut epithelial cells. Inhibition of CYP3A by bioenhancers in gut epithelia will lead to a total increase in drug bioavailability in the serum. Fewer drug molecules will be metabolized by phase I enzymes in the gut and will not be available for phase II conjugation enzymes. This will lead to increased concentrations of untransformed drug passing from gut into the blood and onto other tissues in the body.

Another object of the invention is to reduce variability of oral bioavailability. Reduction of drug biotransformation or increased drug absorption will decrease variability of oral bioavailability to some degree because the increase in bioavailability will begin to approach the theoretical maximum of 100% oral bioavailability. The increase in oral bioavailability will be generally larger in subjects with lower oral bioavailability. The result is a reduction in inter-individual and intra-individual variation. Addition of bioenhancer will reduce inter-individual and intra-individual variation of systemic concentrations of a drug or compound.

Although the primary objective of the bioenhancer is to inhibit CYP3A drug biotransformation in the gut, some biotransformation may be decreased in other tissues as well if the bioenhancer is absorbed into the blood stream. The decrease in biotransformation by other tissues will also increase drug bioavailability. The advantage of targeting a bioenhancer to the gut, however, is that it allows the use of lower systemic concentrations of bioenhancer compared to inhibitors that target CYP3A in the liver. After oral administration of a bioenhancer, concentrations will be highest at the luminal surface of the gut epithelia, not having been diluted by systemic fluids and the tissues of the body. Luminal concentrations that are greater compared to blood concentrations will permit preferential inhibition of CYP3A in gut instead of the liver. Bioenhancers that preferentially inhibit gut CYP3A will also be a particularly effective means of increasing drug bioavailability while minimizing the effects of greater concentrations of bioenhancers in tissues other than the gut.

A Net Increase in Drug Bioavailability Due to a Decrease in the Activity of CYP3A.

The activity of CYP3A is defined as CYP3A catalyzed production of reaction product from CYP3A substrates. Substrates for CYP3A can be naturally occurring substrates or other components such as those listed in Table 1. In addition, some of the CYP3A inhibitors listed in Table 1 have been identified as substrates, as designated in the table. Most likely many if not all of these inhibitors will be shown to be 3A substrates through further research, although allosteric effects are also possible. The catalytic activities of CYP3A, subject to inhibition, include, but are not limited to, dealkyase, oxidase, and hydrolase activities. In addition to the different catalytic activities of CYP3A, different forms of CYP3A exist with a range in molecular weight
(for example, from 51 kD to 54 kD, as shown in Komori et al., *J. Biochem.* 1988, 104:912-16).

TABLE 1

P450 3A substrates	P450 3A inhibitors
Antiarrhythmic	**Antidiabetic**
Amiodarone	Glibenclamide
Lidocaine	Tolbutamide
Quinidine	**Benzodiazepine**
Antiepileptic	Midalazolam*
Ethosuximide	**Calcium channel blocker**
Zonisamide	Diltiazem
Antidepressant	Felodipine
Imipramine	Nicardipine
Tianeptine	Nifedipine*
Benzodiazepine	Verapamil
Clonazepam	**Chemotherapeutic**
Diazepam	Clotrimazole
Triazolam	Erythromycin*
Chemotherapeutics	Fluconazole
Dapsone	Itraconazole
Ifosfamide	Josamycin
Environmental toxins	Ketoconazole
1.6-dinitropyrene	Miconazole
1-nitropyrene	Midecamycin
6-nitrochrysene	Navelbine*
Aflatoxin B1	Primaquine
Benzo(a)pyrene	Triacetylolendomycin*
MOCA[1]	Vinblastine*
PhIP[2]	Vincristine*
Immunosuppressant	Vindesine*
Cyclosporine	**Flavanoids**
FK-506	Benzoflavone
Rapamycin	Kaempferol
Narcotic	Naringenin
Alfentanil	Quercetin
Cocaine	**Steroid hormone**
Codeine	Cortisol*
Ethylmorphine	Ethinylestradiol*
Steroid hormones	Gestodene
17 α-ethynylestradiol	Methylprednisolone
Estradiol	Norgestrel
Flutamide	Prednisolone
Testosterone	Prednisone
Miscellaneous	Progesterone*
1-tetrahydrocannabinol	Tamoxifen*
Acetaminophen	Thiotestosterone
Benzphetamine	**Miscellaneous**
Dextromethorphan	Bromocriptine
Digitoxin	DDEP
Lovastatin	Dihydroergotamine
NOHA[3]	Ergotamine
Retinoic acid	
Selegiline	
Terfenadine	

* Drugs marked * have also been identified as P450 3A substrates.

1 MOCA: 4,4'-Methylene-bis(2-Chloroaniline)

2 PhIP:2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

3 NOHA: N-omega-hydroxy-L-arginine

4 DDEP: 3,5-dicarbethoxy-2,6-dimethyl-4-ethyl-1,4-dihydropyridine

Some bioenhancers reduce CYP3A drug biotransformation by acting either as an inhibitor of CYP3A activity or as a substrate of CYP3A activity. The bioenhancer acting either as the inhibitor or the substrate of CYP3A can act as a competitive, non-competitive, uncompetitive, mixed or irreversible inhibitor of CYP3A drug biotransformation. Additionally, bioenhancer can have properties of being a ligand for P-gp or cytochrome P450 or a ligand for either proteins. Bioenhancers can also include combinations of compounds of different properties. For example, a first compound can act as a P-gp inhibitor while a second compound acts as a CYP3A inhibitor. Bioenhancer can also be bound to the drug being protected, either by covalent bonding or by ionic or polar attractions. Compounds (or drugs) from a number of classes of compounds can be administered with a bioenhancer or can act as a bioenhancer, for example, but not limited to, the following classes: acetanilides, anilides, aminoquinolines, benzhydryl compounds, benzodiazepines, benzofurans, cannabinoids, cyclic peptides, dibenzazepines, digitalis gylcosides, ergot alkaloids, flavonoids, imidazoles, quinolines, macrolides, naphthalenes, opiates (or morphinans), oxazines, oxazoles, phenylalkylamines, piperidines, polycyclic aromatic hydrocarbons, pyrrolidines, pyrrolidinones, stilbenes, sulfonylureas, sulfones, triazoles, tropanes, and vinca alkaloids.

Selection of Compounds for use as Bioenhancers by Reduction of CYP3A Drug Biotransformation

The relative ability of compounds to act as bioenhancers and to increase drug bioavailability can be estimated using *in vitro* and *in vivo* drug biotransformation measurements. *In vivo* measurements of drug bioavailability, such as measuring serum or blood drug concentrations over time, provide the closest measure of total drug systemic availability (bioavailability). *In vitro* assays of CYP3A metabolism and P-pg-transport, as discussed below, indirectly indicate drug bioavailability because CYP3A drug metabolism and P-pg drug transport affect integrated systemic drug concentrations over time. Generally, the ability of a compound being tested to act as a bioenhancer is demonstrated when the addition of the compound to a drug biotransformation assay decreases CYP3A drug biotransformation. Although even a minimally measured increase is all that is required for a compound to be a

bioenhancer, a commercially desirable bioenhancer acting as a CYP3A modulator generally will increase drug bioavailability by at least 10%, preferably by at least 50%, and more preferably by at least 75% of the difference between bioavailability in the presence of the bioenhancer and total availability of the ingested dosage in the absence of the bioenhancer. A sufficient amount of orally administered bioenhancer will provide integrated systemic drug concentrations over time greater than the integrated systemic drug concentrations over time in the absence of bioenhancer.

Compounds that can inhibit enzymes of the P450 3A class can be identified by a variety of bioassays, several of which are set out below.

In vitro CYP3A Assays and Increased Drug Bioavailability

Cell Assays of CYP3A Function and Increased Drug Bioavailability

Cultured cells of either hepatocytes or enterocytes or freshly prepared cells from either liver or gut can be used to determine the ability of a compound to act as a CYP3A inhibitor. Various methods of gut epithelial cell isolation can be used such as the method of Watkins et al., *J. Clin. Invest.* 1985; 80:1029-36. Cultured cells, as described in Schmiedlin-Ren, P. et al., *Biochem. Pharmacol.* 1993; 46:905-918, can also be used. The production of CYP3A metabolites in cells can be measured using high pressure liquid chromatograph (HPLC) methods as described in the following section for microsome assays of CYP3A activity.

Microsome Assays of CYP3A Function and Increased Bioavailability

Microsomes from hepatocytes or enterocytes will be used for CYP3A assays. Microsomes can be prepared from liver using conventional methods as discussed in Kronbach et al., *Clin. Pharmacol. Ther* 1988; 43:630-5. Alternatively, microsomes can be prepared from isolated enterocytes using the method of Watkins et al., *J. Clin. Invest.* 1987; 80:1029-1037. Microsomes from gut epithelial cells can also be prepared using calcium precipitation as described in Bonkovsky, H.L. et al., *Gastroenterology* 1985; 88:458-467. Microsomes can be incubated with drugs and the metabolites monitored as a function of time. In addition the levels of these

enzymes in tissue samples can be measured using radioimmunoassays or western blots.

Isolated microsomes will be used to determine bioenhancer inhibition of CYP3A drug biotransformation. Generally, the drug will be a substrate of CYP3A. The addition of the bioenhancer will decrease the ability of CYP3A to catalyze drug metabolism. Bioenhancers identified in this assay will be inhibitors of CYP3A function and diminish substrate catalysis. The production of metabolites can be monitored using high pressure liquid chromatography systems (HPLC) and identified based on retention times. CYP3A activity can also be assayed by colorimetrically measuring erythromycin demethylase activity as the production of formaldehyde as in Wrighton, et al., *Mol. Pharmacol.* 1985; 28:312-321 and Nash, T., *Biochem. J.* 1953; 55:416-421.

Characteristics of Bioenhancers that Reduce CYP3A Drug Metabolism

Bioenhancers that reduce CYP3A drug metabolism will generally be hydrophobic compounds that can pass across cell membranes and reduce CYP3 drug metabolism in the manner previously indicated.

Preferably the bioenhancer(s) will bind *CYP3*A quickly and inhibit while the drug is passing through the enterocyte. After the bioenhancer diffuses out of the enterocyte, normal *CYP3*A function will return. Reversible and irreversible inhibitors will both have marked effects on gut drug metabolism following oral dosing. After the bioenhancers reach the heart and are distributed throughout the body the concentrations of the bioenhancers will be diluted on future passes through the liver. Concentrations of bioenhancer in the gut lumen are preferably selected to be effective on gut CYP3A metabolism but, due to dilution, to be less active in other tissues.

The amount of bioenhancer used for oral administration can be selected to achieve small intestine luminal concentrations of at least 1/10 of the K_i for CYP3A inhibition of drug metabolism or an amount sufficient to increase systemic drug concentration levels, whichever is less. Alternatively, the amount of an inhibitor of cytochrome P450 3A enzyme that will be used in a formulation can be calculated by various assays that are described in detail below. For example, one such assay measures the conversion of cyclosporine to hydroxylated products in an assay system

containing 100 μg human liver microsomes, 25 μM cyclosporine, and an NADPH regenerating system in 100 μl of 0.1 M sodium phosphate buffer, pH 7.4. The initial inhibitor amount is selected to provide concentrations in the lumen of the small intestine equal or greater than concentrations that reduce the rate of conversion determined by this assay, preferably a rate reduction of at least 10%. While the actual dose of inhibitor in a clinical formulation might be optimized from this initial dosage depending on the results of a clinical trial, the assay as described is sufficient to establish a utilitarian dosage level.

Increased Drug Bioavailability by Inhibition of P-glycoprotein (P-pg)

Increased Drug Absorption By Decreasing P-gp Drug Transport

One embodiment of the present invention further increases bioavailability by increasing net drug absorption in the gut. Traditionally, drug absorption by the gut was considered to be the result of a passive diffusion process. Drugs were thought to diffuse into the gut based on the concentration gradient across the gut epithelial cells. Net drug transport across the gut, however, is the net result of drug influx and back flux, some of which is active drug transport. Drug influx is the flux from lumen to blood. Drug back flux is from blood or epithelium cytoplasm into the lumen. The invention reduces P-gp active drug transport across the luminal membrane to prevent return of drugs absorbed into the cytoplasm of the enterocytes back to the lumen of the gut.

Generally, the invention will reduce P-gp active drug transport in order to increase the net transport of drugs across the gut epithelium. An epithelium exists in a number of different tissue types including, but not limited to, the epithelia of the skin, liver, kidneys, adrenals, intestine, and colon. Such epithelia would be affected by systemic administration of P-gp inhibitors. However, the major effects of the invention will be limited to the gut because of concentration effects resulting from oral delivery.

In embodiments of the invention where the bioenhancer comprises an inhibitor of P-glycoprotein-mediated membrane transport the structure of the bioenhancer can vary widely as long as P-gp-mediated transport is reduced. A number of different

molecules are known to inhibit this transport system, and a number of examples are given below. However, whether a given compound acts as an inhibitor is best determined by activity assays, such as those described below, rather than by reliance on the structure of the molecule.

Because of the many different structures that can act as inhibitors, the oral dosage of inhibitor to be present in the formulation (or elsewise as described below) is best determined empirically, as the dosage will depend on the affinity of the inhibitor for P-gp relative to the drug's affinity for P-gp. There are a number of assays available that allow the desired dosage to be readily determined without requiring clinical trials. While the actual dosage of inhibitor in a clinical formulation might be optimized from this initial dosage depending on results of a clinical trial, the assay as described is sufficient to establish a utilitarian dosage level.

Selection of Compounds for Use as Bioenhancers by Reduction of P-gp Drug Transport/Activity

The relative ability of compounds to act as bioenhancers and to increase drug bioavailability can be estimated using *in vitro* and *in vivo* drug transport measurements. Compounds acting as a bioenhancer will cause a net increase in drug diffusion resulting from a decrease in active P-gp drug transport activity. The activity of P-gp is defined either as ATP dependent membrane transport of a drug or as drug-dependent ATP hydrolysis. P-gp activity or drug flux can be measured using in vitro or in vivo measurements such as, but not limited to, voltage sensitive electrodes or dyes, or chemical sensitive electrodes or dyes, substrate or product analysis, electron microscopy or coupled assays. The form of P-gp used in the assay can vary in molecular weight depending on the species, isoform, amount of glycosylation, and molecular weight assay method. Typically, the molecular weight of the P-gp will be approximately 170 kilodaltons.

The bioenhancer, acting as either the inhibitor or the substrate of P-gp, acts as a competitive, uncompetitive, non-competitive, mixed or irreversible inhibitor of P-gp drug transport. The bioenhancer, as an inhibitor or substrate of P-gp, can be either a transportable or non-transportable ligand of P-gp. The bioenhancer can bind to the P-gp on its lumen accessible surface, cytoplasmic accessible surface or

membrane spanning region. The bioenhancer can be a ligand of P-gp, a ligand of cytochrome P450, or a ligand of both, or any combination of the three types of ligands. For example a bioenhancer can comprise a ligand of P-pg plus a ligand of cytochrome P450 or a ligand of P-gp plus a ligand that binds to both P-gp and cytochrome P450.

Characteristics of bioenhancers that reduce P-gp drug transport

Some of the structural features that have been found for inhibitors of P-glycoprotein-mediated membrane transport include hydrophobic character of the molecule, especially those comprising two co-planar aromatic rings, a positively charged nitrogen group, or a carbonyl group. However, these characteristics are not essential. The bioenhancer can be administered with compounds from classes, such as, but not limited to, aminoacridines, aminoquinolines, anilides, anthracycline antibiotics, antiestrogens, benzofurans, benzhydryl compounds, benzazepines, cannabioids, cephalosporines, colchicine, cyclic peptides, dibenzazepines, epipodophyllotoxins, flavonoids, flavones, imidazole, isoquinolines, macrolides, opioids, phenylalkylamines, phenothiazines, piperazines, piperidines, polyethylene glycols, pyridines, pyridones, pyrimidines, pyrrolidines, quinazolines, quinolines, quinones, rauwolfia alkaloids, retinoids, salicylates, sorbitans, steroids, taxol, triazoles, unsaturated fatty acids, and vinca alkaloids. The bioenhancer can also be made of a compound listed above.

The principal common characteristic of these compounds is that they act as inhibitors of P-gp drug transport. When the bioenhancers are used in sufficient amounts, the activity of P-gp will be reduced; in particular P-gp drug transport back into the intestinal lumen will be reduced. Sufficient amounts would include amounts necessary to increase integrated systemic concentrations over time of the drug used in conjunction with the bioenhancer. The concentration of bioenhancer required to produce a sufficient amount of bioenhancer for inhibition of P-gp drug transport varies with the delivery vehicle used for the bioenhancer and the drug. The luminal concentration of the bioenhancer should be related to the drug's and bioenhancer's relative affinities for P-gp and the drug concentration used. As the affinity of drug

for P-gp increases, the required concentration of the appropriate bioenhancer will increase. Most bioenhancers of commercial application will decrease P-gp drug transport by at least 10%, more preferably by at least 50%, and even more preferably by at least 75%.

Several compounds that are themselves normally thought of as drugs can be used as bioenhancers, including calcium channel blockers, phenothiazines, quinidine, antimalarial agents, antiestrogenic and other steroids, and cyclosporine and other compounds listed in Table 2.

TABLE 2

Classes of P-Glycoprotein Substrates or Inhibitors with Specific Examples

Antiarrhythmics
Amiodarone
Lidocaine
Quinidine

Antibiotics & Antifungals
Cefoperazone
Ceftriaxone
Erythromycin
Itraconazole

Antimalarials & Antiparasites
Chloroquine
Emetine
Hydroxychloroquine
Quinacrine
Quinine

Calcium Channel Blockers
Bepridil
Diltiazem
Felodipine
Nifedipine
Nisoldipine
Nitrendipine
Tiapamil
Verapamil

Cancer chemotherapeutics combination regimens
Actinomycin D
Daunorubicin
Doxorubicin
Mitomylin-C
Taxol
Trimetrexase
Vinblastine
Vincristine

Hormones
Aldosterone
Clomiphene
Cortisol
Dexamethasone
Prednisone
Progesterone
Tamoxifen

Local anesthetics
Bupivacaine

NSAIDs
Aspirin

Phenothiazines

Surfactant/solvents
Cremophor EL
Triton X-100
Tween 80

Tricyclic antidepressants
Desipramine
Trazodone

Miscellaneous
Dipyridamole
Reserpine
Cyclosporine
Colchicine
FK-506
Liposomes
Quercetin
SDZ PSC-833
SDZ 280-446
Terfenadine
Tumor Necrosis Factor
Vitamin A

Any bioassay that determines whether a given compound has the inhibition characteristics required of a bioenhancer can be used to identify compounds that can be used in the practice of the present invention. A number of such assays are set out below.

In vitro P-pg Assays for Bioavailability

Everted Gut Assays

Everted intestine can be prepared by methods known in the art Hsing *et al.* Gastrobentsrology 1992; 102:879-85). In these studies rat small intestines turned "inside out" (i.e. the mucosal (or luminal) surface turned outside and the serosal surface inside) are bathed in a drug containing solution with and without the addition of the bioenhancer. The serosal surface of the small intestine is bathed in a solution that is periodically monitored or changed for the purpose of drug or bioenhancer measurement. For instance the everted rat small intestines can be bathed in a physiological saline solution loaded with Rhodamine 123 (Rh123) and the flux of Rh 123 monitored into the serosal solution. The addition of a bioenhancer in this set-up will increase Rh 123 transport into the serosal solution. An increase in drug or Rh 123 bioavailability will be determined as follows:

$$\frac{X(100)}{Y}$$

where Y is the initial rate of Rh 123 transport, and X is the initial rate of rhodamine transport in the presence of a bioenhancer. The initial rates will be determined as a linear relationship between time and Rh 123 concentration in the luminal solution. Alternatively, the serosal side of rat small intestines is bathed with the drug or bioenhancer of interest and the mucosal solution is monitored, as described in Hsing *et al.* (1992).

Selection of a P-gp Inhibitor Based on Cell Growth Assays

This assay will be used to select candidate bioenhancers. Cells cultured with cytotoxic agents that are known P-gp transport substrates will be grown as controls in the absence of either drug or bioenhancer. The $appK_i$ (apparent inhibition constant) for cell growth by drugs will be determined by varying the drug

concentration in the culture medium. The $appK_i$ will be expressed as the concentration of drug required to produce 50% inhibition of cell growth. Cells will also be grown in the presence of drug and bioenhancer. The bioenhancer will act to shift the $appK_i$ to lower drug concentrations necessary for inhibition of cell growth. Cells with MDR can be used in this assay as described in Hait, W. N., et al., Biochemical Pharmacology 1993, 45:401-406. The method sections of Hait, W.N., et al. (1993) are herein incorporated by reference. Preferred bioenhancers will decrease the $appK_i$ for a drug by at least 2 times, more preferably by at least 3 times, and even more preferably by at least 6 times.

Rhodamine (Rh 123) Cellular Assay of P-gp Drug Transport and Drug Bioavailability

Rh 123 can be used in a cellular assay to monitor the bioavailability of drugs. Rh 123 transported by P-gp in this system acts as a drug, where P-gp pumps the Rh 123 out of the cell. Single cells or a population of cells can be monitored for the Rh 123 fluorescence which is indicative of P-gp transport. The cell types used will contain a P-gp transporter from a MDR strain such as those listed in Nielsen and Skovsgaard, *Biochimica et Biophysica Acta* 1993; 1139:169-183 and herein incorporated by reference. Cells are loaded with Rh 123 in the presence of 15 nanograms per ml to 500 nanograms per ml of Rh 123 in a physiologically compatible buffer such as 3-N-morpholinopropanesulfonic acid (MOPS) with the suitable concentrations of sodium, potassium, and calcium chloride and an energy source. The cells are loaded with Rh 123 for 30 - 60 minutes depending on the temperature (37° or room temperature). The loaded cells are then washed and resuspended in buffer free of Rh 123. The efflux of Rh 123 can be determined using a fluorimeter. In the absence of any bioenhancer Rh 123 will be pumped out of the cell due to the action of P-gp, leading to a reduced amount of Rh 123 fluorescence from the cell.

Addition of a P-gp substrate or inhibitor either by preincubation after the cells have been washed with Rh 123 free buffer or during the efflux of Rh 123 from the cell will cause retention of Rh 123 within the cell. Retention of Rh 123 in the cell will be caused by the addition of a bioenhancer. Increased drug bioavailability is

defined as the increase in Rh 123 retention within the cell. Compounds that increase Rh 123 retention are bioenhancers.

Rh 123 retention in the absence of a bioenhancer will be determined by total Rh 123 cell fluorescence minus background Rh 123 cell fluorescence. An increase in drug bioavailability due to the addition of the bioenhancer will be the percentage increase in Rh 123 fluorescence retention as described by:

$$\frac{X}{Y}(100)$$

where X equals Rh 123 fluorescence in the presence of the bioenhancer minus the background Rh 123 fluorescence and Y equals the Rh 123 fluorescence in the absence of the bioenhancer minus the background Rh 123 fluorescence.

The background Rh 123 fluorescence can be measured in a variety of ways including, but not limited to, the residual amount of Rh 123 fluorescence at the end of the experiment, the residual amount of Rh 123 fluorescence remaining based on an extrapolation of first order rate kinetics describing the efflux of Rh 123 from the cell, the residual amount of Rh 123 fluorescence in the presence of a sufficient amount of membrane detergents such as triton or digitonin, or the amount of Rh 123 fluorescence in the presence of a potassium-valinomycin clamp.

The addition of both a second drug and a bioenhancer to the Rh 123 assay will not necessarily cause an increased amount of Rh 123 retention compared to the presence of either the bioenhancer alone or the second drug alone. This is because Rh 123 retention can already be very high due to the second drug or bioenhancer concentration. Extra retention due to the addition of either the second drug or the bioenhancer can be difficult to measure above the signal for Rh 123 in the presence of the second drug or bioenhancer alone. However, once it has been determined that the drug (or second drug alone) increases Rh 123 fluorescence, i.e. decreases Rh 123 efflux, it can be assumed that the drug (or second drug alone) is transported by the P-gp transport system.

Vesicle Assays of P-pg Activity and Drug Bioavailability

A particularly preferred assay uses brush border membranes. Brush border membrane vesicles are prepared from the small intestine by methods known in the

art, such as, Hsing, S. *et al.*, *Gastroenterology* 1992; 102:879-885. The vesicles will be assayed for the presence of P-gp by using monoclonal antibodies directed to P-gp either using SDS page gel electrophoresis and western blotting techniques or using immunochemistry and electromicroscopy. Vesicles containing P-gp will be used for drug transport assays.

Drug transport assays consist of measuring the transport of drugs into the vesicles in an adenosine triphosphate (ATP) dependent fashion. Uptake of the drug in the presence of ATP will be monitored using fluorescence or absorbance techniques, for instance using Rh 123 as the fluorescent drug transported into the interior of the vesicle. Radioactively labeled drugs can also be used to monitor drug transport into the interior of the vesicle using a filter wash system. The addition of ATP will induce the transport of the drug into the vesicle and will increase drug transport compared to passive diffusion of the drug into the vesicle interior. Addition of non-hydrolyzable analogs of ATP such as ATP gamma S or adenosine monophosphate para-nitrophenol (AMP-PNP) will not produce an ATP dependent influx of drug into the vesicle. Thus, the introduction of a non-hydrolyzable nucleotide can be used as a control to monitor whether drug transport has actually occurred due to ATP hydrolysis from the P-gp transport system.

The addition of a bioenhancer to this assay system using a fluorescent drug or a radioactive drug and monitoring its uptake, will reduce the uptake of the drug into the interior of the vesicle with the addition of ATP. This reduction in drug transport represents an increase of the bioavailability of the drug. The vesicles transporting drugs in an ATP dependent fashion are oriented with the cystolic face of the P-gp accessible to the ATP. It is these vesicles that hydrolyze the ATP and transport the drug into the interior of the vesicle. The interior of the vesicle in turn corresponds to the luminal surface or the apical membrane of the brush border cells. Thus, transport into the lumen of the vesicle or interior of the vesicle corresponds to transport into the lumen of the gut. A decrease in the transport of the lumen of the vesicle is the equivalent of increasing net drug absorption and increasing the drug bioavailability.

P-gp ATPase Assays of P-gp Activity and Drug Bioavailability

P-gp molecules can be isolated in vesicles suitable for measuring ATP'ase activity. P-gp ATP'ase activity will be measured in the presence of other types of ATP'ase inhibitors, such as, but not limited to, sodium potassium ATPase inhibitors (ouabain and vanadate), mitochondrial ATP'ase inhibitors such as oligomycin, and alkaline phosphatase inhibitors. The ATP'ase assays will also be conducted in the absence of sodium and potassium to eliminate background sodium and potassium ATP'ase activity. ATP'ase activity will be measured as ATP'ase activity dependent on the presence of a drug such as daunomycin. ATP'ase activity will be measured using ATP or hydrolyzable ATP analogs such para-nitrophenolphosphate. The production of product will be monitored using phosphate assay procedures of those of Yoda, A. and Hokin, L., *Biochem. Biophys. Res. Comm. 1970; 40:880-886* or by monitoring phosphatase activity as recognized in the literature.

An increase in P-gp ATPase activity due to the addition of a drug is recognized as an increase in drug bioavailability. P-gp molecules located in the brush border membrane vesicles are oriented so the cytosolic portion of the molecule finds and hydrolyzes ATP. It is these P-gp molecules that will give rise to the drug dependent ATPase activity. Bioenhancer that is able to stimulate the ATPase activity will be able to compete with the drug for the P-gp transport system. Such bioenhancers will decrease P-gp drug transport due to their increased ability to stimulate P-gp activity. Bioenhancers can also inhibit drug dependent P-gp ATP'ase activity without stimulating P-gp ATP'ase activity thus, inhibiting drug transport.

Another manner of determining the amount of bioenhancer appropriate for an oral formulation is based on the K_i of the specific inhibitor (for whichever binding is being measured). An appropriate amount of inhibitor is one that is sufficient to produce a concentration of the bioenhancer in the lumen of the gut of the animal of at least 0.1 times the K_i of the bioenhancer.

In all of these cases, the goal of selecting a particular concentration is increased bioavailability of the pharmaceutical compound that is being administered. Thus, a desirable goal is to provide integrated systemic concentrations over time of the pharmaceutical compound in the presence of the inhibitor that is greater than the

integrated systemic concentrations over time of the pharmaceutical compound in the absence of the inhibitor by at least 10% of the difference between bioavailability in its absence and complete oral bioavailability. Preferred is attaining of "complete bioavailability," which is 100% systemic bioavailability of the administered dosage.

Screening Assay for Bioenhancers

In summary, the various techniques described above for screening candidate bioenhancer compounds for activity by assaying for inhibition in the gut of a mammal of activity of a cytochrome P450 enzyme or of transport by P glycoprotein are all generally useful as methods of identifying compounds that are useful for increasing bioavailability of a drug in a mammal. In all of these assays, the best bioenhancers are those compounds selected from the candidate compounds being tested that best inhibit either transport or enzymatic destruction (preferably the latter) of a tested drug in the gut of the mammal (either by direct testing *in vivo* or by a test that predicts such activity). When testing for inhibition of activity of a cytochrome enzyme, assays that detect inhibition of members of a cytochrome P450 3A family (for a particular mammal, particularly human) are preferred. Although *in vivo* assays are preferred, because of the direct relationship between the measurement and gut activity, other assays, such as assays for inhibition of cytochrome P450 activity in isolated enterocytes or microsomes obtained from enterocytes of the mammal in question or for inhibition of cytochrome P450 in a tissue or membrane from the gut of said mammal, are still useful as screening assays. The same ordering of preferred screening assays (i.e., *in vivo* being preferred over *in vitro*) is also preferred for screening of inhibition of P-gp transport. Screening by assaying for both inhibitions is preferred, with inhibition of cytochrome P450 activity generally being more important than that of P-gp-mediated transport.

Coadministration and Delivery of Bioenhancers

Increase in Drug Bioavailability with Coadministration of a Bioenhancer and a Drug

The present invention will increase the bioavailability of the drug in the systemic fluids or tissues by co-administering the bioenhancer with a drug. "Co-

administration" includes concurrent administration (administration of the bioenhancer and drug at the same time) and time varied administration (administration of the bioenhancer at a time different from that of the drug), as long as both the bioenhancer and the drug are present in the gut lumen and/or membranes during at least partially overlapping times. Systemic fluids or tissues refer to drug concentration measured in blood, plasma or serum, and other body fluids or tissues in which drug measurements can be obtained.

Delivery Vehicles Provide For Coadministration

Coadministration can vary in the type of delivery vehicle. The bioenhancer and the drug can use different delivery vehicles such as, but not limited to, time release matrices, time release coatings, companion ions, and successive oral administrations. Alternatively, the drug and the bioenhancer can be formulated with different coatings possessing different time constants of bioenhancer and drug release. The use of bioenhancers also applies to epithelia tissues other than the gut. Aspects of the invention used in the gut are appropriately used in other types of epithelia. For example, CYP 3A enzymes and P-glycoprotein have also been demonstrated in the skin and bioenhancers used in transdermal formulations would increase drug bioavailability to systemic fluids and tissues. Such applications are included as part of the invention herein because of inhibition by bioenhancers of CYP 3A enzymes and P-glycoprotein in epithelia other than the gut.

Formulations of Bioenhancers

In some embodiments, the bioenhancer comprises a cytochrome-P450-3A-inhibiting compound and a separate P-glycoprotein-inhibiting compound. In other cases, the bioenhancer comprises a single compound that inhibits both CYP3A and P- glycoprotein, or just one of the two processes. The bioenhancer is preferably present as a counter ion of the pharmaceutical compound in order to ensure that the bioenhancer is present at maximum concentration in the presence of the drug that it is protecting.

The cytochrome P450 3A family of enzymes and the P-gp transporting protein both have a wide range of substrates, and thus potential inhibitors, as exemplified by

the variety of structures present in compounds that can act as inhibitors as set forth above.

The invention is carried out in part by formulating an oral pharmaceutical composition to contain a bioenhancer. This is accomplished in some embodiments by admixing a pharmaceutical compound, a pharmaceutical carrier, and a bioenhancer comprising an inhibitor of P-glycoprotein-mediated membrane transport or an inhibitor of a cytochrome P450 3A enzyme, the bioenhancer being present in sufficient amount to provide integrated systemic concentrations over time of the compound as measured by AUC's greater than the integrated systemic concentrations over time of the compound in the absence of the composition when the pharmaceutical composition is administered orally to an animal being treated with the pharmaceutical composition. A pharmaceutical carrier increases drug solubility or protects drug structure or aids in drug delivery or any combination thereof.

Pharmaceutical compositions produced by the process described herein are also part of the present invention.

In addition to use with new formulations, the present invention can also be used to increase the bioavailability of the active compound of an existing oral pharmaceutical composition. When practiced in this manner, the invention is carried out by reformulating the existing composition to provide a reformulated composition by admixing the active compound with a bioenhancer comprising an inhibitor of a cytochrome P450 3A enzyme or an inhibitor of P-glycoprotein-mediated membrane transport, the bioenhancer being present in sufficient amount to provide integrated systemic concentrations over time of the compound when administered in the reformulated composition greater than the integrated systemic concentrations over time of the compound when administered in the existing pharmaceutical composition. All of the criteria described for new formulations also apply to reformulation of old compositions. In preferred aspects of reformulations, the reformulated composition comprises all components present in the existing pharmaceutical composition plus the bioenhancer, thus simplifying practice of the invention, although it is also possible to eliminate existing components of formulations because of the increase in bioavailability. Thus, the invention also covers reformulated compositions that

contain less than all components present in the existing pharmaceutical composition plus the bioenhancer. However, this invention does not cover already existing compositions that contain a component which increases bioavailability by mechanisms described in this specification (without knowledge of the mechanisms), should such compositions exist.

Traditional formulations can be used with bioenhancers. Optimal bioenhancer doses can be determined by varying the coadministration of bioenhancer and drug in time and amount dependent fashion and monitoring bioavailability. Once the optimal bioenhancer dose is established for a drug the formulation (bioenhancer, drug and formulation composition(s)) is tested to verify the increased bioavailability. In the case of time or sustained release formulations it will be preferred to establish the optimal bioenhancer dose using such formulations from the start of the bioavailability experiments.

EXAMPLE - QUANTITATION OF BIOENHANCER IN VIVO

Cyclosporin (CYA) Bioavailability in the Absence and Presence of Ketoconazole (KC) As A Bioenhancer.

1. General Design of Methodology:

Six male/female healthy volunteers served as subjects for the procedure. Pre-procedure laboratory tests, physical examinations, and consent was obtained at least five days prior to the procedure date.

The procedure was performed in two phases. During the initial phase (I), baseline oral and intravenous pharmacokinetic parameters were established. Phase II consisted of ketoconazole administration and oral/intravenous pharmacokinetic procedures. Each phase followed identical procedures: after an overnight fast, subjects were admitted for initial CYA pharmacokinetics procedures. The order of CYA route of administration was randomized during phase I, and the same order was maintained in phase II. After the insertion of an indwelling catheter, each subject received an oral or intravenous dose of CYA with 5mL blood draws obtained at 0, 15, 30, 45, and 60 minutes, then 2, 3, 4, 5, 6, 8, 10, 12, 14, and 24 hours (total

volume of blood: 300mL). During the intravenous infusion, contralateral catheters were inserted. Intravenous CYA was administered over 2.5 hours by AVI infusion pump. After completing the i.v. infusion, the infusion catheter was removed. Subjects either returned to the testing location in the morning for each 24 hour blood draw or stayed overnight in the testing location.

During phase I, subjects received oral or i.v. CYA (8mg/kg and 2.5mg/kg, respectively) and followed the above procedures (Day 1). After a three day washout period (Day 5), subjects received CYA i.v. or p.o. depending on initial randomization. Blood samples were drawn as described above. When subjects returned to the testing location for the 24 hour blood draw (Day 6), they were given KC 200mg and instructed to take one tablet daily at 10PM for eight days. The last dose of ketoconazole was taken the night of procedure day 14, just before the last pharmacokinetic procedure day.

In study sections designed to evaluate Ketoconazole's effects (Phase II) on both intravenous and oral CYA metabolism, dosing of the bioenhancer and the drug (CYA) were separated by approximately 10 hours.

During phase II (Day 11), subjects were admitted to the testing location after an overnight fast for further CYA pharmacokinetic procedures. Subjects received either a single oral dose of CYA at a reduced dose of 2mg/kg or intravenous CYA (0.6mg/kg) depending on the previous randomization schedule. Blood samples were drawn as described above. After a three day washout period, on Day 15, subjects received the oral (2mg/kg) or i.v. (0.6mg/kg) dose, again depending on the initial randomization schedule. Blood samples were drawn as described above.

In phase II, in healthy volunteers, dosing of CYA in the presence of the enhancer was reduced from that administered when no bioenhancer was present for safety considerations.

The total volume of blood drawn for this procedure was 380mL (pre/post labs and pharmacokinetic procedures.

2. SAMPLE AND DATA ANALYSIS:

Whole blood samples were assayed for CYA and metabolites (AM1, AM4N, AM9, AM1c9) by HPLC. Pharmacokinetic parameters, including bioavailability, were obtained from data and compared for differences between baseline (-KC) and inhibition (+KC).

3. SUBJECT SELECTION CRITERIA:

a. Healthy adult volunteers were used to minimize interpatient variation in response to drug administration, which can occur with hepatic or renal dysfunction, and to minimize risks associated with drug administration.

b. Eight healthy male or female subjects were recruited for this procedure to assure that at least six subjects completed both phases.

c. INCLUSION CRITERIA:

* >than 18 years of age

* Weight not more than 10% above or below the ideal body weight for age, height and weight. (Metropolitan Life Insurance Co. tables)

* Good health on the basis of history and physical exam.

* No history of cardiovascular, renal, hepatic, gastrointestinal, respiratory, hematologic disease, or other diseases which could affect the distribution, absorption, metabolism, or excretion of either procedure drugs.

* Have laboratory tests within normal limits.

* Ability to provide written and informed consent.

d. EXCLUSION CRITERIA:

* Use of any drugs, including both prescription and chronic over-the-counter medications within one week of the procedure. Specifically, use of antacids, H_2-antagonists, or other agents known to decrease KC absorption or interactions with CYA.

* Participation in experimental drug procedure within one month preceding the procedure.

* History of hypersensitivity of KC, azole-antifungal agents, or CYA.

* History of myxedema, hyperthyroidism, hepatic disease, hepatitis, alcohol, or recreational drug use, cardiac arrhythmias, seizures, tobacco, or any other condition which can alter drug metabolism, absorption, or distribution.

4. SUBJECT RECRUITMENT:

Subjects were recruited from the population in and around a university campus. Potential subjects were screened for their fitness to participate in the procedure within 5 days prior to procedure date. The screening procedure included the following: medical and drug histories, physical exam and baseline laboratory procedures (hemoglobin, hematocrit, RBC, WBC, differential, platelets, potassium, chloride, bicarbonate, serum creatinine, BUN, glucose, albumin, total bilirubin, alkaline phosphatase, AST, ALT, cholesterol, HOL, LOL, and a urine pregnancy test when female volunteers were used).

5. SPECIFIC PROCEDURES:

a. At least five days prior to the procedure date, a 40mL blood sample was drawn from each subject for baseline laboratory procedures as stated above. At this time a history and physical exam was performed.

b. The procedure was divided into two phases. During phase I, baseline CYA pharmacokinetics after intravenous and oral dosing were established. During phase II, subjects were given eight days of KC therapy, and post-KC intravenous and oral CYA pharmacokinetic procedures were performed. Subjects were randomized to receive an oral or i.v. dose as the initial route of administration and followed this randomization throughout the procedure.

Day 1: Subjects were admitted to the testing location at 07:00 after an overnight fast from 22:00 the previous evening. An indwelling polyethylene catheter with a teflon obturator was inserted aseptically into the forearm vein for blood withdraw. Immediately prior to drug administration, a 5mL blood sample was collected. CYA was then administered orally (8mg/kg) or intravenously (2.5mg/kg

over 2.5 hours) based on body weight. Five milliliter blood samples were drawn at the following times post during administration: 15, 30, 45, 60 minutes and 2, 3, 4, 5, 6, 8, 10, 12, 14, and 24 hours. Subjects returned to the testing location the following day (Day 2) for the 24 hour blood draw (peripheral venipuncture). Whole blood was analyzed for parent drug and metabolites. Some samples were frozen prior to extraction. Breakfast, lunch, a light snack, and dinner were supplied during all days spent in the testing location. All subjects were given oral CYA with chocolate milk to ensure adequate absorption and to minimize variability in absorption. Subjects were allowed to drink water freely.

Day 5: After a three day wash-out period, subjects were again admitted to the testing location, and identical procedures were followed as described above in Day 1 except that subjects received CYA by the alternate route of administration not used previously on Day 1. Subjects receiving CYA by the i.v. route had the i.v. catheter removed after the infusion was completed. Blood samples were drawn as described above.

Day 6: When returning for the 24 hour blood draw, subjects received a 100mg dose of ketoconazole (half-dose) and were observed for one hour, the other 100mg being taken at 10pm that evening. Each subject was instructed to take one 200mg tablet each evening at 10pm with food. Subjects were instructed to take KC for a total of eight days. The last dose of KC was taken on Day 14.

Day 11: The procedure went forward as described for Day 1 with the exception of CYA dosing. Subjects received an oral dose of 2mg/kg of CYA or an i.v. dose of 0.6mg/kg over 2.5 hours. Blood samples were drawn as previously described. An identical diet was provided to minimize changes in CYA absorption.

Day 15: After the three day wash-out period, subjects were admitted to the testing location and received the final CYA i.v. or oral dose. Blood samples were drawn as previously described. The 24 hour blood draw included an additional 40mL to perform post-procedure laboratory tests. Prior to discharge, each subject underwent a physical exam and history to detect any adverse reactions due to drugs or catheter insertion.

Since CYA absorption can be highly variable, especially with diet content, food outside of the procedure diet was not permitted. All meals (breakfast, lunch, snack, dinner) were supplied on all procedure days.

F. RESULTS

KC inhibited the metabolism of cyclosporine (CYA), resulting in elevated CYA levels. CYA as a single dose was administered orally and intravenously, pre- and post-KC treatment to 6 normal healthy volunteers. The mean pre-KC bioavailability (F) was 22.2% compared to 62.0% post-KC ($P<0.003$). Utilizing $F_{hepatic}=1-ER$, where $ER=CL_{IV}/$hepatic blood flow (1.28L/hr/kg), F can be broken down into its components: $F_{hepatic}xF_{abs}\ xF_{gut}$. $F_{abs}xF_{gut}$ increased significantly post-KC (68.5%) compared to pre-KC (26.2%) ($P<0.006$), whereas F_H changed minimally, $90\%^{post\text{-}KC}$ vs $86\%^{pre\text{-}KC}$ ($P=0.10$). CYA is well absorbed (>62%) by the gut under these conditions.

The interaction between CYA and ketoconazole is also expressed graphically in Figure 1. Subject CYA levels were measured after IV administration of CYA pre-KC treatment (pre-IV) and post-KC treatment (post-IV). Subject CYA levels were also measured after oral administration of CYA pre-KC treatment (pre-PO) and post-KC treatment (post-PO). The increase in CYA concentration was greatest post-KC treatment of orally administered CYA.

This example demonstrates the effect of intestinal P450 enzymes on the bioavailability of CYA. It shows that intestinal P450 enzymes are important determinants of the bioavailability of CYA and other agents which undergo significant metabolism by P450 enzymes.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WE CLAIM:

1. A method for increasing bioavailability of an orally administered hydrophobic pharmaceutical compound, which comprises:

orally administering said pharmaceutical compound to a mammal in need of treatment with said compound concurrently with a bioenhancer comprising an inhibitor of a cytochrome P450 3A enzyme or an inhibitor of P-glycoprotein-mediated membrane transport, said bioenhancer being present in sufficient amount to provide bioavailability of said compound in the presence of said bioenhancer greater than bioavailability of said compound in the absence of said bioenhancer.

2. The method of Claim 1, wherein said bioenhancer comprises an inhibitor of a cytochrome P450 3A enzyme and said inhibitor is a hydrophobic molecule.

3. The method of Claim 1, wherein said bioenhancer comprises an inhibitor of a cytochrome P450 3A enzyme and said inhibitor is present at a concentration in the gut of said mammal equal to or greater than a concentration of said bioenhancer that reduces conversion of cyclosporine to hydroxylated products by 10%, compared to controls, in an assay system containing 100 μg human liver or enterocyte microsomes, 25 μM cyclosporine, and an NADPH regenerating system in 100 μl of 0.1 M sodium phosphate buffer, pH 7.4.

4. The method of Claim 1, wherein said amount is sufficient to produce a concentration of said bioenhancer in the lumen of the gut of said mammal of at least 0.1 times said K_i or apparent K_i of the bioenhancer.

5. The method of Claim 1, wherein bioavailability in the presence of said inhibitor is greater than bioavailability of said compound in its absence by at least 10% of the difference between bioavailability in its absence and complete oral bioavailability.

6. The method of Claim 1, wherein said bioenhancer comprises an inhibitor of P-glycoprotein-mediated membrane transport and said inhibitor is a hydrophobic molecule comprising two co-planar aromatic rings, a positively charged nitrogen group, or a carbonyl group.

7. The method of Claim 1, wherein said bioenhancer comprises an inhibitor of P-glycoprotein-mediated membrane transport and said inhibitor is present at concentrations equal to or greater than concentrations that reduce transport of Rh 123 by P-glycoprotein in brush border membrane vesicles or P-gp containing cells by 10%.

8. The method of Claim 1, wherein said bioenhancer comprises a cytochrome-P450-3A-inhibiting compound and a separate P-glycoprotein-inhibiting compound.

9. The method of Claim 1, wherein said bioenhancer comprises a single compound that inhibits both cytochrome P450 3A and P glycoprotein.

10. The method of Claim 1, wherein said bioenhancer is present as a counter ion of said pharmaceutical compound.

11. The method of Claim 1, wherein said bioenhancer is covalently bound to said pharmaceutical compound.

12. The method of Claim 1, wherein said pharmaceutical compound comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazapine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide, dibenzazepine, digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, pyridine, pyridone, pyrimidine, pyrrolidine, pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid, salicylate, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, or vinca alkaloid.

13. The method of Claim 1, wherein said bioenhancer comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazapine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide, dibenzazepine, digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, flavone, flavonoid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, polyethylene glycol, pyridine, pyridone, pyrimidine, pyrrolidine, pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid, salicylate, sorbitan, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, unsaturated fatty acid, or vinca alkaloid.

14. The method of Claim 1, wherein said inhibitor is selected from said group consisting of the compounds listed in Tables 1 and 2.

15. The method of Claim 1, wherein said bioenhancer reduces inter-individual variability of said bioavailability of said pharmaceutical compound.

16. The method of Claim 1, wherein said bioenhancer reduces intra-individual variability of said bioavailability of said pharmaceutical compound.

17. A method of formulating an oral pharmaceutical composition, which comprises:

admixing a pharmaceutical compound, a pharmaceutical carrier, and a bioenhancer comprising an inhibitor of P-glycoprotein-mediated membrane transport or an inhibitor of a cytochrome P450 3A enzyme, said bioenhancer being present in sufficient amount to provide bioavailability of said pharmaceutical compound in the presence of said bioenhancer greater than the bioavailability of said pharmaceutical compound in the absence of said bioenhancer when said pharmaceutical composition is administered orally to an mammal.

18. The method of Claim 17, wherein said bioenhancer comprises an inhibitor of a cytochrome P450 3A enzyme and said inhibitor is a hydrophobic molecule.

19. The method of Claim 17, wherein said bioenhancer comprises an inhibitor of a cytochrome P450 3A enzyme and said inhibitor is present in an amount sufficient to provide a lumen concentration equal to or greater than a concentration of said bioenhancer that reduces conversion of cyclosporine to hydroxylated products by 10% in an assay system containing 100 μg human liver or enterocyte microsomes, 25 μM cyclosporine, and an NADPH regenerating system in 100 μl of 0.1 M sodium phosphate buffer, pH 7.4.

20. The method of Claim 17, wherein said bioenhancer comprises an inhibitor of P-glycoprotein-mediated membrane transport and said inhibitor is a hydrophobic molecule comprising two co-planar aromatic rings, a positively charged nitrogen group, or a carbonyl group.

21. The method of Claim 17, wherein said bioenhancer comprises an inhibitor of P-glycoprotein-mediated membrane transport and said inhibitor is present in the gut of said mammal at a concentration equal to or greater than a concentration that reduces transport of Rh 123 by P-glycoprotein in brush border membrane vesicles or P-gp-containing cells by 10%.

22. The method of Claim 17, wherein said amount is sufficient to produce a concentration of said bioenhancer in the lumen of the gut of said mammal of at least 0.1 times said K_i or apparent K_i of said bioenhancer.

23. The method of Claim 17, wherein said inhibitor comprises a cytochrome-P450-3A-inhibiting compound and a separate P-glycoprotein-inhibiting compound.

24. The method of Claim 17, wherein said inhibitor comprises a single compound that inhibits both cytochrome P450 3A and P glycoprotein.

25. The method of Claim 17, wherein said bioenhancer is present as a counter ion of said pharmaceutical compound.

26. The method of Claim 17, wherein said bioenhancer is covalently bound to said pharmaceutical compound.

27. The method of Claim 17, wherein said pharmaceutical compound comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazapine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide, dibenzazepine, digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, pyridine, pyridone, pyrimidine, pyrrolidine, pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid, salicylate, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, or vinca alkaloid.

28. The method of Claim 17, wherein said bioenhancer comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazapine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide, dibenzazepine, digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, flavone, flavonoid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, polyethylene glycol, pyridine, pyridone, pyrimidine, pyrrolidine, pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid, salicylate, sorbitan, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, unsaturated fatty acid, or vinca alkaloid.

29. The method of Claim 17, wherein said inhibitor is selected from said group consisting of the compounds listed in Tables 1 and 2.

30. A pharmaceutical composition produced by the process of Claim 17.

31. A method of increasing bioavailability of the active compound of an existing oral pharmaceutical composition, which comprises:

reformulating said existing composition to provide a reformulated composition by admixing said active compound with a bioenhancer comprising an inhibitor of cytochrome P450 3A enzyme or an inhibitor of P-glycoprotein-mediated membrane transport, said bioenhancer being present in sufficient amount to provide bioavailability of said pharmaceutical compound when administered in said reformulated composition greater than said bioavailability of said pharmaceutical compound when administered in said existing pharmaceutical composition.

32. The method of Claim 31, wherein said reformulated composition comprises all components present in said existing pharmaceutical composition plus said bioenhancer.

33. The method of Claim 31, wherein said reformulated composition contains less than all components present in said existing pharmaceutical composition plus said bioenhancer.

34. A reformulated pharmaceutical composition produced by the process of Claim 31.

35. A method of identifying a compound useful for increasing bioavailability of a drug in a mammal, which comprises:

screening candidate compounds by assaying for inhibition of a cytochrome P450 enzyme or of transport by P glycoprotein by said candidate compounds in the gut of said mammal, and

selecting from said candidate compounds a compound or compounds that inhibit either said enzymes or said transport in said gut.

36. The method of Claim 35, wherein said assaying is for inhibition of said cytochrome P450 enzyme in said gut.

37. The method of Claim 35, wherein said cytochrome P450 enzyme is a member of a cytochrome P450 3A family.

38. The method of Claim 35, wherein said assaying measures inhibition of cytochrome P450 activity in isolated enterocytes or microsomes obtained from enterocytes or microsomes of said mammal.

39. The method of Claim 35, wherein said assaying measures inhibition of cytochrome P450 in a tissue or membrane from the gut of said mammal.

40. The method of Claim 35, wherein said assaying measures inhibition of cytochrome P450 *in vivo* in the gut of said mammal.

41. The method of Claim 35, wherein said screening comprises assaying for both inhibitions.

42. The method of Claim 35, wherein said assaying measures inhibition of P glycoprotein transport in isolated cells from the gut of said mammal.

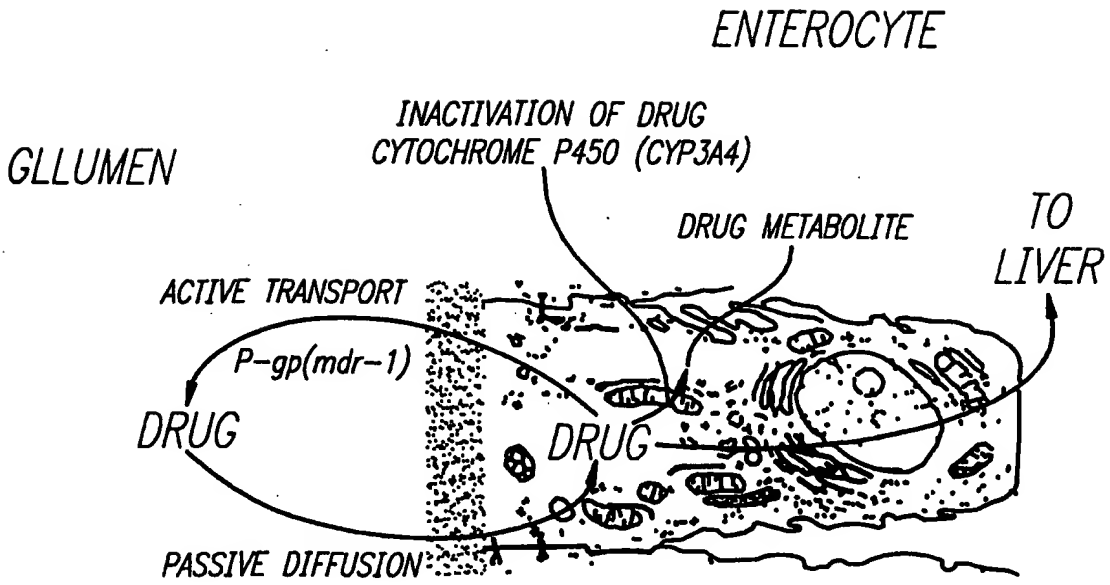
43. The method of Claim 35, wherein said assaying measures inhibition of P glycoprotein transport in a tissue or membrane from the gut of said mammal.

44. The method of Claim 35, wherein said assaying measures inhibition of P glycoprotein transport *in vivo* in the gut of said mammal.

45. A method for increasing bioavailability of a topically administered hydrophobic pharmaceutical compound, which comprises:

topically administering said pharmaceutical compound to a mammal in need of treatment with said compound concurrently with a bioenhancer comprising an inhibitor of a cytochrome P450 3A enzyme or an inhibitor of P-glycoprotein-mediated membrane transport, said bioenhancer being present in sufficient amount to provide bioavailability of said compound in the presence of said bioenhancer greater than bioavailability of said compound in the absence of said bioenhancer.

FIG. 1


ENTEROCYTE
INACTIVATION OF DRUG
CYTOCHROME P450 (CYP3A4)
GLLUMEN
DRUG METABOLITE
TO
LIVER
ACTIVE TRANSPORT
P-gp(mdr-1)
DRUG
DRUG
PASSIVE DIFFUSION

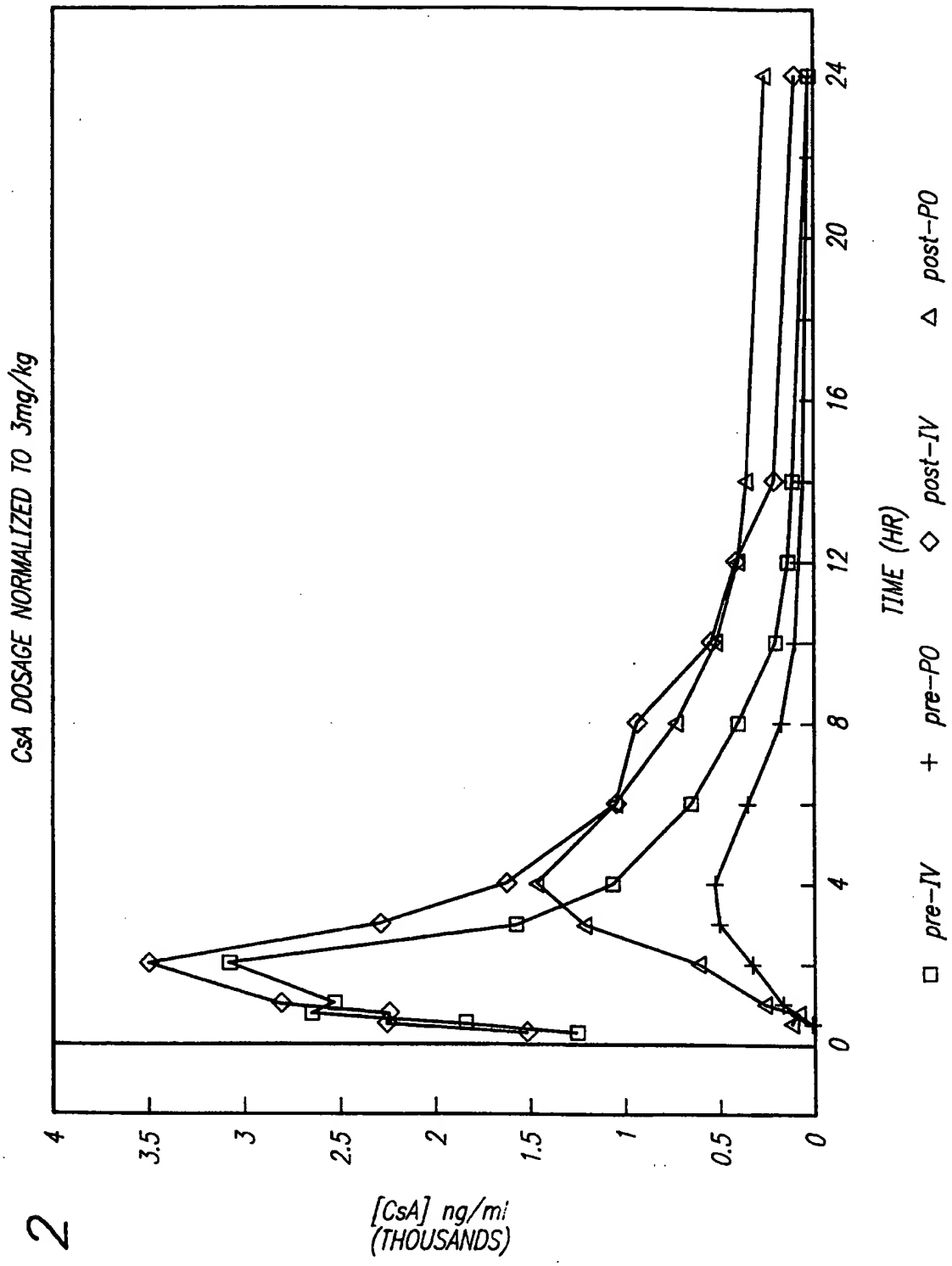

CsA DOSAGE NORMALIZED TO 3mg/kg
[CsA] ng/ml (THOUSANDS)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
4
8
12
16
20
24
TIME (HR)
□ pre-IV
+ pre-PO
◇ post-IV
△ post-PO

FIG. 2

INTERNATIONAL SEARCH REPORT

Inter ɔnal Application No
PCT/US 95/00347

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K45/06 A61K38/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TRANSPLANTATION (BALTIMORE), 51 (2). 1991. 365-370., FIRST M R ET AL 'CYCLOSPORINE DOSE REDUCTION BY KETOCONAZOLE ADMINISTRATION IN RENAL TRANSPLANT RECIPIENTS' see abstract ---	1-45
X	CLIN. TRANSPLANT., 1992, 6/2 (134-138), DENMARK, SRIDHAR N. ET AL 'Influence of concomitant medication on cyclosporine dosage and blood concentrations in renal allograft recipients' see abstract --- -/--	1-45

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 May 1995	31. 05. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Leherte, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLECULAR PHARMACOLOGY, 41 (6). 1992. 1047-1055., PICHARD L ET AL 'Effect of corticosteroids on the expression of cytochromes P450 and on cyclosporin A oxidase activity in primary cultures of human hepatocytes' see abstract ---	1-45
X	ARCH. INTERN. MED., 1993, 153/17 (1970-1976), USA, BACIEWICZ A.M. ET AL 'Ketoconazole and fluconazole drug interactions' see page 1975, column 3, paragraph 1 ---	1-45
X	CLIN. TRANSPLANT., 1992, 6/3 I (141-153), DENMARK, CHAN G.L. ET AL 'Drug interactions with cyclosporine: Focus on antimicrobial agents' see abstract ---	1-45
X	INT. J. CLIN. PHARMACOL. THER. TOXICOL., 1992, 30/12 (555-570), GERMANY, ALBENGRES E. ET AL 'Cyclosporin and ketoconazole, drug interaction or therapeutic association?' see abstract ---	1-45
X	BR. J. DERMATOL. SUPPL., 1992, 126/39 (14-18), UNITED KINGDOM, BACK D.J. ET AL 'Azoles, allylamines and drug metabolism' see page 17 ---	1-45
X	CLIN. PHARMACOKIN., 1990, 19/4 (319-332), NEW ZEALAND, YEE G.C. ET AL 'Pharmacokinetic drug interactions with cyclosporin (Part I)' see abstract ---	1-45
X	BR J CLIN PHARMACOL, 28 (2). 1989. 166-170., BACK D J ET AL 'COMPARATIVE EFFECTS OF TWO ANTIMYCOTIC AGENTS KETOCONAZOLE AND TERBINAFINE ON THE METABOLISM OF TOLBUTAMIDE ETHYNYLESTRADIOL CYCLOSPORIN AND ETHOXYCOUMARIN BY HUMAN LIVER MICROSOMES IN-VITRO' see abstract ---	1-45

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INTERNATIONAL SEARCH REPORT

Inter. ..nal Application No
PCT/US 95/00347

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LANCET, 2 (8673). 1989. 1198-1201., FIRST M R ET AL 'CONCOMITANT ADMINISTRATION OF CYCLOSPORIN AND KETOCONAZOLE IN RENAL TRANSPLANT RECIPIENTS' see abstract	1-45
X	AM J OPHTHALMOL, 113 (6). 1992. 687-690., DE SMET M D ET AL 'COMBINED USE OF CYCLOSPORINE AND KETOCONAZOLE IN THE TREATMENT OF ENDOGENOUS UVEITIS' see abstract	1-45
X	DRUG METAB. DISPOS., 18/5, 1990, 595-606, PICHARD, LYDIANE ET AL 'Cyclosporin A drug interactions. Screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes' see abstract	1-45
X	PHARMACOL. TOXICOL. (COPENHAGEN), 66, 1990, 49-52, HENRICSSON, S. ET AL 'Cyclosporin metabolism in human liver microsomes and its inhibition by other drugs' see abstract	1-45
X	REV INFECT DIS, SEP-OCT 1989, 11 (5) P691-7, UNITED STATES, SANDS M ET AL 'Interactions of cyclosporine with antimicrobial agents [see comments]' see abstract	1-45

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US95/00347

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: cl. 1-29,31-33,35-45
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Althoug claims are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-45
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Reason: In view of the large number of compounds which are defined by the w ording of the claims, the search has been performed on the general idea and compounds mentioned in the example of the description (Chapter III, point 3.6 of the PCT Search Guidelines)

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.